Rolle von SGK1 in der Salzempfindlichkeit von Blutdruck und der Glukoseaufnahme: Studien in Knockoutmäusen

Role of SGK1 in Salt Sensitivity of Blood Pressure and Peripheral Glucose Uptake: Studies in Knockout Mice

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Krishna Murthy Boini

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1. Berichterstatter:	Prof. Dr. F. Lang
2. Berichterstatter:	Prof. Dr. P. Ruth
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CONTENTS

ABBREVIATIONS

ASDN: Aldosterone sensitive distal nephron

B.W.: Body weight

CCD: Cortical collecting duct

Dexa: Dexamethasone

DOCA: Deoxycorticosterone acetate

EDTA: Ethylene diamine tetra acetate

ENaC: Epithelial sodium channel

F: Fructose

GFR: Glomerular filtration rate

H_2O_2: Hydrogen peroxide

HCl: Hydrochloric acid

HFD: High fat diet

I.P.: Intraperitoneal

Kg: Kilogram

KO: Knockout

MAP: Mean arterial pressure

MCD: Medullary collecting duct

Mg: Milligram

$MgCl_2$: Magnesium Chloride

MR: Mineralocorticoid receptor

NaCl: Sodium Chloride

PDK1: Phosphoinositide dependent kinase 1

PI-3K: Phosphatidylinositide-3-kinase

PKB: Protein kinase B

PPARγ: Peroxisome proliferator activated receptor γ

ROMK: Renal outer medullary potassium channel

SD: Standard diet

SGK1: Serum and glucocorticoid inducible kinase 1

SPL: Spironolactone

TGF: Tissue growth factor

SUMMARY

Excess salt intake increases blood pressure particularly during states of hyperinsulinism and insulin resistance. Insulin is presumably effective through activation of ENaC. Excess salt intake further decreases peripheral glucose uptake thus impairing glucose tolerance. Stimulation of both, the epithelial Na^+ channel ENaC and of cellular glucose uptake involves phosphatidylinositide 3-kinase (PI-3K) which signals through protein kinase B (Akt/PKB) and all three members of the serum and glucocorticoid inducible kinase (SGK) family of kinases SGK1, SGK2 and SGK3. All three kinases have been previously shown to modify a variety of transporters including ENaC and the glucose transporter SGLT1. To explore the role of SGK1 in salt sensitive hypertension and peripheral glucose uptake, experiments were performed in male or female SGK1 knockout mice (*sgk1*$^{-/-}$) and their wild type littermates (*sgk1*$^{+/+}$) which were subjected to standard diet, high-fat diet, high fructose diet or dexamethasone treatment and allowed free access to either tap water (control-salt) or 1% saline (high-salt).

Under control diet fluid intake, blood pressure, urinary flow rate and urinary Na^+, K^+, Cl^- excretion were similar in *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$mice, plasma aldosterone concentration was however significantly higher in *sgk1*$^{-/-}$ (1.22 ± 0.18 ng/ml) than in *sgk1*$^{+/+}$mice (0.57 ± 0.11 ng/ml). Under standard diet, high-salt-intake (1% NaCl in drinking water for 25 days) increased fluid intake, urinary flow rate and urinary Na^+, K^+, Cl^- excretion similarly in *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$mice without significantly altering blood pressure. High-fat-diet alone (17 weeks) did not significantly alter fluid intake, urinary flow rate, urinary Na^+, K^+, Cl^- excretion or plasma aldosterone levels, but increased plasma insulin, total cholesterol and triglyceride concentrations as well as systolic blood pressure to the same extent in both genotypes. Additional high-salt-intake (1% NaCl in drinking water for 25 days) on top of high-fat-diet did not affect hyperinsulinemia nor hyperlipidemia but increased fluid intake, urinary flow rate and urinary NaCl excretion significantly more in *sgk1*$^{-/-}$ than in *sgk1*$^{+/+}$mice. Furthermore, in animals receiving high fat diet, additional salt intake increased blood pressure only in *sgk1*$^{+/+}$mice (132 ± 3 mmHg) but not in *sgk1*$^{-/-}$mice (120 ± 4 mmHg). Renal SGK1 protein abundance of *sgk1*$^{+/+}$ mice was significantly elevated following high fat diet.

During 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 neither 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. 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, $p < 0.05$) following combined fructose and high salt intake. Renal SGK1 transcript levels of *sgk1*$^{+/+}$ mice were significantly elevated following fructose diet. 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.

Prior to dexamethasone treatment, the fluid intake, urinary flow rate, urinary Na^+, K^+ and Cl^- excretion, plasma electrolyte and glucose concentrations as well as blood pressure were similar in *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ mice. Dexamethasone treatment (3mg/kg, b.w. i.p. for 14 days) did not significantly alter renal Na^+, K^+ and Cl^- excretion, but it tended to decrease renal Ca^{2+} excretion in *sgk1*$^{+/+}$ mice but significantly increased renal Ca^{2+} excretion in *sgk1*$^{-/-}$ mice and significantly decreased renal phosphate excretion in *sgk1*$^{+/+}$ mice. Dexamethasone treatment significantly increased fasting blood glucose concentrations in both genotypes. Dexamethasone treatment significantly increased blood pressure in *sgk1*$^{+/+}$ mice, an effect significantly blunted in *sgk1*$^{-/-}$ mice. The subsequent replacement of the tap drinking water with saline increased the fluid intake, urinary flow rate and urinary NaCl excretion in both genotypes but increased plasma K^+ concentration only in *sgk1*$^{-/-}$ mice. Saline loading increased blood pressure in both, *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ mice and dissipated the difference between genotypes.

Intraperitoneal injection of glucose (3g/kg/body-weight) 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 animals (164 ± 23 mg/dl). 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-weight). [glucose]p,max was in *sgk1*$^{-/-}$mice insensitive to high-salt and significantly higher than in control *sgk1*$^{+/+}$mice. Uptake of ^{3}H-deoxy-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. According to Western blotting, high-salt decreased and

DOCA (35 mg/kg/body-weight) increased SGK1 protein abundance in skeletal muscle and fat tissue of *sgk1*$^{+/+}$ mice. Transfection of HEK293 cells with active ^{S422D}SGK1 but not inactive ^{K127N}SGK stimulated phloretin-sensitive glucose uptake.

In conclusion, lack of SGK1 protects against the hypertensive effects of combined high-fat-diet/high-salt-intake or high fructose/high salt diet and mediates the salt sensitive peripheral glucose uptake. 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 those 5% of the common population carrying the SGK1 gene variant. The observations suggest that SGK1 plays a critical role in the hypertensive effect of hyperinsulinism. As a gain of function gene variant of SGK1 could simultaneously increase blood pressure and body mass index, SGK1 may indeed be one of the signalling molecules contributing to metabolic syndrome or syndrome X, a condition characterized by the coincidence of several disorders including hypertension, obesity, insulin resistance and hyperinsulinemia. Metabolic syndrome shares several attributes of Cushing´s syndrome, but does not require increased plasma cortisol levels. Instead, the disorder may be caused by inappropriate activity of downstream signaling elements which could well include the serum and glucocorticoid inducible kinase SGK1.

Zusammenfassung

Exzessive Salzaufnahme erhöht den Blutdruck, besonders unter Hyperinsulinämie und Insulinresistenz. Insulin wirkt vermutlich über Aktivierung des epithelialen Natriumskanals ENaC. Weiterhin vermindert exzessive Salzaufnahme die periphere Glucoseaufnahme und beeinträchtigt somit die Glucosetoleranz. Bei der Stimulation des epithelialen Natriumkanals ENaC als auch der zellulären Glucoseaufnahme ist die Phosphatidylinositol-3-kinase (PI-3K) beteiligt, deren Signalkaskade die Proteinkinase B (Akt/PKB) und alle drei Mitglieder der Serum- und Glukokortikoid-induzierbaren Kinase (SGK)-Familie SGK1, SGK2 und SGK3 einschließt. Für alle drei Kinasen wurde kürzlich gezeigt, dass sie eine Vielzahl von Transportern regulieren, darunter ENaC und den Glucosetransporter SGLT1. Um die Rolle von SGK1 bei salzabhängiger Hypertonie und peripherer Glucoseaufnahme zu untersuchen, wurden Experimente in SGK1-Knockout-Mäusen (*sgk1*$^{-/-}$) und den Wildtyp-Geschwistern (*sgk1*$^{+/+}$) durchgeführt, die einer Standard-Diät, einer fettreichen Diät, einer hohen Fructose-Diät und Dexamethasonbehandlung unterzogen wurden und die freien Zugang entweder zu Leitungswasser (Kontrolle) oder zu 1%iger NaCl-Lösung (hohe Salzzufuhr) hatten.

Unter Kontrollbedingungen waren Flüssigkeitsaufnahme, Blutdruck, Harnflussrate und Ausscheidung von Na^+, K^+ und Cl^- mit dem Urin bei *sgk1*$^{-/-}$- und *sgk1*$^{+/+}$-Mäusen ähnlich, die Plasma-Aldosteron-Konzentration war jedoch bei *sgk1*$^{-/-}$-Mäusen (1.22 ± 0.18 ng/ml) signifikant höher als bei *sgk1*$^{+/+}$-Mäusen (0.57 ± 0.11 ng/ml). Unter Standarddiät erhöhte eine hohe Salzzufuhr (1% NaCl im Trinkwasser für 25 Tage) Flüssigkeitsaufnahme, Harnflussrate und Urin-Ausscheidung von Na^+, K^+ und Cl^- bei *sgk1*$^{-/-}$- und *sgk1*$^{+/+}$-Mäusen in ähnlicher Weise ohne den Blutdruck signifikant zu ändern. Eine alleinige fettreiche Diät über 17 Wochen änderte Flüssigkeitsaufnahme, Harnflussrate, Urin-Ausscheidung von Na^+, K^+ und Cl^- oder die Plasma-Aldosteron-Spiegel nicht signifikant, aber erhöhte bei beiden Genotypen die Plasmainsulinspiegel im gleichen Ausmaß, die Plasmakonzentrationen von Gesamtcholesterin und Triglyceriden ebenso wie den systolischen Blutdruck. Eine zur fettreichen Diät zusätzlich verabreichte Salzzufuhr (1% NaCl im Trinkwasser für 25 Tage) beeinflusste weder Hyperinsulinämie noch Hyperlipidämie, aber Flüssigkeitsaufnahme, Harnflussrate und Urin-Ausscheidung von Na^+ und Cl^-. Letztere waren bei *sgk1*$^{-/-}$-Mäusen im Vergleich zu *sgk1*$^{+/+}$-Mäusen signifikant erhöht. Diese Behandlung erhöhte nur bei *sgk1*$^{+/+}$-Mäusen den Blutdruck (132 ± 3 mmHg) nicht jedoch bei

sgk1$^{-/-}$-Mäusen (120 ± 4 mmHg). Die Proteinexpression der SGK1 in der Niere war bei *sgk1*$^{+/+}$-Mäusen nach einer fettreichen Diät signifikant erhöht.

Ein Zusatz von 10% Fructose zum Trinkwasser erhöhte Flüssigkeitsaufnahme und Harnflussrate bei beiden Genotypen und änderte die Urin-Ausscheidung von Na^+, K^+ and Cl^- nicht. Eine zusätzliche Salzbelastung in Form einer NaCl-reichen Diät (4% NaCl) änderte Flüssigkeitsaufnahme und Harnvolumen nicht signifikant, aber erhöhte deutlich die Urin-Ausscheidung von Na^+ und Cl^- und erreichte bei *sgk1*$^{-/-}$-Mäusen signifikant höhere Werte als bei *sgk1*$^{+/+}$-Mäusen. Der Blutdruck war bei *sgk1*$^{+/+}$- und *sgk1*$^{-/-}$-Mäusen unter Kontrolldiät oder alleiniger Fructosezugabe ähnlich und war nur bei *sgk1*$^{+/+}$-Mäusen (115 ± 1 vs. 103 ± 1 mmHg) unter einer kombinierten Fructose- und hohen Salzaufnahme erhöht. Eine akute intravenöse Insulininfusion unter gleichzeitiger Glucoseinfusion bewirkte in *sgk1*$^{+/+}$-Mäusen eine Antinatriurese. Dieser Effekt war bei *sgk1*$^{-/-}$-Mäusen signifikant abgeschwächt. Die Beobachtungen zeigten, dass SGK1 bei Insulin-vermittelter Natriumretention und bei salzabhängigen hypertensiven Effekten durch hohe Fructoseaufnahme eine zentrale Rolle spielt.

Vor Behandlung mit Dexamethason waren Flüssigkeitsaufnahme, Harnflussrate, Urin-Ausscheidung von Na^+, K^+ and Cl^-, Plasmaelektrolyt- und Glucosekonzentrationen ebenso wie Blutdruck bei *sgk1*$^{-/-}$- und *sgk1*$^{+/+}$-Mäusen ähnlich. Behandlung mit Dexamethason (3mg/kg Körpergewicht, i.p. für 14 Tage) veränderte die renale Na^+-, K^+- und Cl^--Ausscheidung nicht signifikant, aber verringerte die renale Ca^{2+}-Ausscheidung bei *sgk1*$^{+/+}$-Mäusen tendenziell, bei *sgk1*$^{-/-}$-Mäusen kam es allerdings zu einer signifikanten Erhöhung der renalen Ca^{2+}-Ausscheidung. Die Dexamethason-Behandlung erhöhte die Blutglucosekonzentration nach Fasten bei beiden Genotypen signifikant. Darüberhinaus, erhöhte die Dexamethason-Behandlung den Blutdruck bei *sgk1*$^{+/+}$-Mäusen signifikant, dieser Effekt war bei *sgk1*$^{-/-}$-Mäusen signifikant abgeschwächt. Im folgenden wurde das Trinkwasser mit Salz versetzt, was Flüssigkeitsaufnahme, Harnflussrate und NaCl-Ausscheidung im Urin bei beiden Genotypen erhöhte, die Plasma-K^+-Konzentration jedoch nur bei *sgk1*$^{-/-}$-Mäusen erhöhte. Die Salzlast erhöhte den Blutdruck sowohl bei *sgk1*$^{-/-}$- als auch bei *sgk1*$^{+/+}$-Mäusen und es gab keinen Unterschied zwischen beiden Genotypen.

Während eines intraperitonealen Glucosetoleranztests (3g/kg/Körpergewicht) in *sgk1*$^{+/+}$-Mäusen stieg die Plasmaglucosekonzentration vorübergehend an und erreichte dabei bei Mäusen unter NaCl-reichen Diät (281 ± 39 mg/dl) signifikant höhere Spitzenwerte als bei den Kontrolltieren (164 ± 23 mg/dl). DOCA (35 mg/kg/KG) alleine veränderte in der Kontrollgruppe der *sgk1*$^{+/+}$-Mäuse die Spitzenwerte nicht signifikant, aber verminderte diese

signifikant unter gleichzeitiger NaCl-reichen Diät. Dieser Effekt konnte durch Spironolacton (50 mg/kg/KG) umgekehrt werden. Die Spitzenwerte waren bei *sgk1*$^{-/-}$-Mäusen unabhängig von hoher Salzdiät signifikant höher als in der Kontrollgruppe der *sgk1*$^{+/+}$-Mäuse. Die Aufnahme von ^{3}H-desoxy-glucose in den Skelettmuskel und ins Fettgewebe war bei *sgk1*$^{-/-}$-Mäusen signifikant geringer als bei *sgk1*$^{+/+}$-Mäusen und bei *sgk1*$^{+/+}$-Mäusen durch eine hohe Salzdiät verringert. Im Western-Blot verminderte eine hohe Salzzufuhr die Proteinexpression der SGK1 im Skelettmuskel und im Fettgewebe von *sgk1*$^{+/+}$-Mäusen, DOCA erhöhte diese. Die Transfektion von HEK293-Zellen mit aktivem ^{S422D}SGK1, aber nicht inaktivem ^{K127N}SGK stimulierte die Phloretin-abhängige Glucoseaufnahme.

Schlussfolgernd kann man sagen, dass ein Mangel an SGK1 vor hypertensiven Effekten unter kombinierter hoher Fettdiät und hoher Salzzufuhr oder unter hoher Fructosezufuhr und hoher Salzdiät schützt und die salzabhängige periphere Glucoseaufnahme moduliert. Die aktuellen Beobachtungen ergeben Hinweise für die SGK1-abhängige Steigerung des Blutdrucks und stellen damit möglicherweise einen Erklärungsansatz für den erhöhten Blutdruck bei jenen 5% der Gesamtbevölkerung mit einem SGK1-Genpolymorphismus dar. Die Beobachtungen lassen vermuten, dass die SGK1 eine entscheidende Rolle im Hinblick auf hypertensive Effekte unter Hyperinsulinämie spielt. Da eine Überfunktion der SGK1-Genvariante gleichzeitig den Blutdruck und den Körpermassenindex BMI erhöhen könnte, ist SGK1 vermutlich in der Tat eines der Signalmoleküle, das zum metabolischen Syndrom oder Syndrom X führt, ein Zustand, der durch das Zusammenwirken mehrerer Funktionsstörungen charakterisiert ist, darunter Bluthochdruck, Übergewicht, Insulinresistenz und Hyperinsulinämie. Das metabolische Syndrom hat einige Merkmale mit dem Cushing-Syndrom gemeinsam, aber weist keinen erhöhten Plasma-Cortisolspiegel auf. Stattdessen wird die Störung vermutlich von unangemessen erhöhter Aktivität von "downstream"-Signalelementen verursacht, die durchaus die Serum- und Glukokortikoid-induzierbare Kinase SGK1 miteinschließen könnte.

I. INTRODUCTION

Serum and glucocorticoid inducible kinase (SGK1) was originally isolated in a differential screen searching for glucocorticoid-induced transcripts in a mammary tumor cell line (Webster et al., 1993). It was found to be induced within 30 minutes, either by glucocorticoids or serum and in both mammary epithelial cells and fibroblasts (Webster et al., 1993; Webster et al., 1993a). This induction persisted in mammary cells (Webster et al., 1993), whereas in fibroblasts it was transient and returned to normal after 4 hours of induction. Sgk mRNA is extremely unstable in fibroblasts (Webster et al., 1993a), a finding that subsequently has been confirmed in other cells (Kumar et al., 1999). SGK (specifically SGK1, as there are two closely related paralogs referred to as SGK2 and SGK3) is remarkable in that it is tightly regulated by numerous signaling molecules via its expression levels, intrinsic kinase activity and subcellular localization. These properties allow it to integrate numerous signaling pathways. It is best known as a kinase that acts as a cell survival molecule in different situations and that is able to regulate numerous membrane transport and channel proteins.

SGK1 is a member of the AGC family of serine/ threonine protein kinases. Its catalytic domain is approximately 45–60% homologous to the catalytic domains of other serine/threonine protein kinases such as Akt/protein kinase B or protein kinase A (PKA) (Webster et al., 1993). In accordance with its name, the expression of SGK1 in the kidney is sensitive to glucocorticoids (Alvarez de la Rosa et al., 2003; Brennan et al., 2000; Shigaev et al., 2000). Based on studies in rat kidney, Alvarez de la Rosa et al. (2003) suggested that approximately half of the basal level of renal SGK1 expression is maintained by glucocorticoids. Moreover, aldosterone upregulates the expression of SGK1 in the ASDN (Firestone et al., 2003; Pearce 2003; Verrey et al., 2003; Vallon et al., 2005; Bhargava et al., 2004). Whereas aldosterone can stimulate the expression of SGK1, the latter has to be phosphorylated for activation. Phosphorylation of SGK1 involves signaling cascades that include phosphatidylinositol 3-kinase and the 3-phospho- inositide-dependent kinases PDK1 and PDK2 (Biondi et al., 2001; Kobayashi et al., 1999; Park et al., 1999; Tong et al., 2004). Reported 3-phosphoinositide-dependent kinase-dependent activators of SGK1 include insulin and growth factors such as insulin-like growth factor (Kobayashi et al., 1999; Hayashi et al., 2001; Perrotti et al., 2001). Further, aldosterone itself may promote

SGK1 activation by a phosphatidylinositol 3-kinase-dependent mechanism (Tong et al., 2004; Blazer Yost et al., 1999; Wang et al., 2001). Other pathways independent of phosphatidylinositol (3,4,5)-trisphosphate such as cell–cell and matrix interactions and phosphorylation by PKA have also been found to activate SGK1 (Perrotti et al., 2001; Shelly et al., 2002). Thus, SGK1 may integrate the influences of aldosterone and factors like insulin or PKA-activator arginine vasopressin on renal NaCl reabsorption (Wang et al., 2001; Faletti et al., 2002).

SGK1 is induced by a very large spectrum of stimuli distinct from glucocorticoids and serum (Table 1). These include aldosterone (Chen et al., 1999; Naray-Feges-Toth et al., 1999), cell shrinkage (Waldegger et al., 1997; Warntges et al., 2002), cell swelling (Rozansky et al., 2002), TGF-β (Waldegger et al., 1999; Lang et al., 2000; Fillon et al., 2002; Khan et al., 2005), ischemic injury of the brain (Imaizumi et al., 1994; Nishida et al., 2004), neuronal excitotoxicity (Hollister et al., 1997), memory consolidation (Tsai et al., 2002), chronic viral hepatitis (Fillon et al., 2002), DNA damaging agents (You et al., 2004), vitamin D3 (Akutsu et al., 2001), psychophysiological stress (Murata et al., 2005), iron (Marzullo et al., 2004), glucose (Khan et al., 2005), endothelin-1 (Khan et al., 2005), granulocyte-macrophage colony–stimulating factor (GM-CSF) (Cowling and Birnboim 2000), fibroblast growth factor (FGF) (Mizuno and Nishida 2001), platelet-derived growth factor (PDGF) (Mizuno and Nishida 2001), phorbolesters (Mizuno and Nishida 2001), follicle-stimulating hormone (FSH) (Alliston et al., 1997), sorbitol (You et al., 2004; Leong et al., 2003), heat shock (You et al., 2004; Leong et al., 2003), oxidative stress (You et al., 2004; Leong et al., 2003), UV irradiation (You et al., 2004; Leong et al., 2003), and p53 (Firestone et al., 2003; Maiyar et al., 1997; You et al., 2004; Maiyer et al., 1996).

1.1 TABLE 1: Stimuli that cause induction of SGK1 expression

Stimuli	**Cell or tissue type**	**Reference(s)**
Serum	Ubiquitous	Webster et al., 1993
Glucocorticoids	Ubiquitous	Webster et al., 1993
Aldosterone	ASDN	Chen et al., 1999; Naray-Feges-Toth et al., 1999
Cell shrinkage	HepG2, MDCK,	Waldegger et al., 1997;

	neuroblastoma	Warntges et al., 2002
Cell swelling	A6 cells	Rozansky et al., 2002
TGF-β	U937, HepG2, intestine, fibroblasts, endothelial cells	Waldegger et al., 1999; Lang et al., 2000; Fillon et al., 2002; Khan et al., 2005
Chronic viral hepatitis	Liver	Fillon et al., 2002
Ischemic injury of brain	Brain	Imaizumi et al., 1994; Nishida et al., 2004
Neuronal excitotoxicity	Brain, glial cells	Hollister et al., 1997
Memory consolidation	Brain, hypocampus	Tsai et al., 2002
DNA damage	Fibroblasts	You et al., 2004
1a,25-dihydroxyvitamin D3	Squamous cell carcinoma	Akutsu et al., 2001
Psychophysiological stress	Brain, heart, kidney	Murata et al., 2005
Iron	Intestine	Marzullo et al., 2004
Glucose	Endothelial cells	Khan et al., 2005
Endothelin-1	Endothelial cells	Khan et al., 2005
Granulocyte-macrophage colony–stimulating factor	Granulocytes	Cowling and Birnboim 2000
Fibroblast growth factor	NIH 3T3 cells	Mizuno and Nishida 2001
Platelet-derived growth factor	NIH 3T3 cells	Mizuno and Nishida 2001
Phorbolesters	NIH 3T3 cells	Mizuno and Nishida 2001
Follicle-stimulating hormone	Rat ovarian granulosa cells	Alliston et al., 1997
Sorbitol	NMuMg mammary epithelial cells	You et al., 2004; Leong et al., 2003
UV radiation	NMuMg mammary epithelial cells	You et al., 2004; Leong et al., 2003
Heat shock	NMuMg mammary epithelial cells	You et al., 2004; Leong et al., 2003
Oxidative stress/H2O2	NMuMg mammary epithelial cells	You et al., 2004; Leong et al., 2003
PPARγ	ASDN	Hong et al., 2003
p53	Mammary epithelial cells	Firestone et al., 2003; Maiyar et al., 1997; You et al., 2004

1.2 The SGK1 Isoforms SGK2 and SGK3

There are two paralogs of SGK1, SGK2 and SGK3/CISK, which share 80% amino acid identity with SGK1 and with each other in their catalytic domains (Kobayashi et al., 1999; Liu et al., 2000; Dai et al., 1999). The three enzymes differ in the region N-terminal of the C-terminal catalytic domain: SGK2 contains a relatively short N terminus (98 amino

acids), with no discernable domain, whereas SGK3 has a longer N terminus (162 amino acids) comprising a phox homology (PX) domain (Xu et al., 2001). PX domains were originally found as conserved domains in the $p40^{phox}$ and $p47^{phox}$ subunits of the neutrophil NADPH oxidase (phox) superoxide-generating complex (Ponting 1996). These domains are part of many proteins involved in intracellular protein trafficking, such as the sorting nexins (Worby and Dixon 2002). PX domains are phosphoinositide-binding domains that appear to be important for localization of these proteins to membranes (especially endosomes) enriched in phosphoinositides. In this respect, these domains resemble other domains such as the pleckstrin homology (PH), FYFE, FERM and ENTH domains. PKB/Akt contains a PH domain in its N terminus, which is important for PKB/Akt activation by phosphoinositide-3 kinases (PI-3Ks). This domain enables the colocalization with the 3-phosphoinositide-dependent protein kinase-1 (PDK-1), which is known to phosphorylate and activate PKB/Akt. Similarly, SGK3's PX domain is involved in SGK3 localization and activity: It is necessary for phosphoinositide binding, endosomal localization, and proper kinase activity (Xu et al., 2001; Liu et al., 2000). Moreover, structural studies indicate that it may play a role in dimerization of the kinase (Xing et al., 2004). With respect to their physiological role(s), it has been shown *in vitro* that both the SGK2 and SGK3 enzymes have the same phosphorylation consensus as SGK1 (and PKB/Akt), namely R-X-R-X-X-(S/T) (Kobayashi et al., 1999). It is likely, however, that other factors, such as surrounding amino acids, subcellular localization, or cofactors are important for the specificity of and functional differences between the enzymes. For example, in *Xenopus* A6 cells, only SGK1 and not the coexpressed PKB modulates the activity of the epithelial Na^+ channel (ENaC) (Artega et al., 2005). The role of SGK2 has mainly been studied in heterologous expression systems such as *Xenopus laevis* oocytes or HEK293 cells and with respect to numerous transport and channel proteins. These studies revealed that SGK2 can stimulate the activity of K^+ channels such as the voltage- gated K^+ channel Kv1.3 (Gamper et al., 2002; Henke et al., 2004), Na^+,K^+-ATPase (Henke et al., 2002), KCNE1 (Embark et al., 2003), ENaC (Friedrich et al., 2003), the glutamate transporter EEAT4 (Bohmer et al., 2004), and the glutamate receptors GluR6 (Strutz-Seebohm et al., 2005) and GluR1 (Strutz-Seebohm et al., 2005a). All of these transport proteins are also stimulated in the same cellular systems by SGK1, SGK3, and/or PKB; hence, the physiological relevance of these findings has to be considered with caution. To define more precisely the role of SGK2, it will be necessary to

carry out additional studies, using more relevant cell or animal systems and knocking down SGK2 by either RNA interference protocols or by gene inactivation. SGK3/CISK, which is better characterized than SGK2, was identified in a screen for antiapoptotic genes (Liu et al., 2000) and found to act downstream of the PI-3K pathway and in parallel with PKB/Akt. Moreover, it was demonstrated to phosphorylate and inhibit Bad (a proapoptotic protein) and FKHRL1, a proapoptotic transcription factor. Knockout (KO) mice have been generated; these mice are viable and fertile and have normal Na^+ handling and glucose tolerance, as opposed to the KO mice of SGK1 or PKB/Akt2 (McCormick et al., 2004; Garofalo et al., 2003; Wulff et al., 2002; Cho et al., 2001). However, they display after birth a defect in hair follicle development, a defect preceded by disturbances in the β-catenein/Lef1 gene regulation (McCormick et al., 2004). Like SGK2, SGK3 has been implicated in the regulation of numerous transporters and channels, including K^+ channels (Gamper et al., 2002; Henke et al., 2004; Embark et al., 2003), Na^+,K^+-ATPase (Henke et al., 2002), the glutamate transporter EEAT1 (Boehmer et al., 2003), the cardiac voltage-gated Na^+ channel SCN5A (Boehmer et al., 2003), ENaC (Friedrich et al., 2003), Na^+-dicarboxylate cotransporter 1 (Boehmer et al., 2004), the chloride channel ClCa/barttin (Embark et al., 2004), the epithelial Ca^{2+} channel TRPV5 (Embark et al., 2004), the Na^+-phosphate cotransporter NaPi1b (Palmada et al., 2004), the amino acid transporter ASCT2 (Palmada et al., 2005), GluR1, and GluR6 (Strutz-Seebohm et al., 2005; Strutz-Seebohm et al., 2005a). For the same reasons mentioned above for SGK2, additional studies on SGK3 will be necessary to evaluate the physiological relevance of these findings.

1.3. Regulation of SGK1 by Phosphorylation

Many AGC kinases contain in their catalytic domain and in the C-terminal region conserved phosphorylation motifs. The motif in the catalytic domain, situated on the so-called activation loop (A-loop), was first identified in PKB/Akt and was shown to be a target of PDK-1 (Alessi et al., 1997; Stokoe et al., 1997). Hence, PKB/Akt is a kinase regulated by an upstream kinase, which depends on the PI-3K system. As outlined above, both PKB/Akt and PDK-1 contain a PH domain, enabling them to interact with phosphoinositides [phosphatidylinositol-(3,4)-biphosphate and phosphatidylinositol-(3,4,5)-triphosphate, or PtdIns(3,4)P2 and PtdIns(3,4,5)P3, respectively] generated by PI-

3K. The PH domain recruits both kinases to membranes and allows PDK-1 to phosphorylate PKB/Akt. This activation is very fast (approximately 2 minutes after stimulation with insulin or growth factor) (Vanhaesebroeck and Alessi 2000). PDK-1-dependent phosphorylation sites are also found in PKA (Cheng et al., 1998), PKCζ (Chou et al., 1998; Le Good et al., 1998), p70S6K (Alessi et al., 1997; Pullen et al., 1998), and the SGK paralogs (Kobayashi and Cohen 1999; Park et al., 1999). In SGK1 it is Thr256 in the A-loop of the catalytic domain. However, although SGK1 is also dependent on the PI-3K pathway and on PDK-1 activity, the mode of activation of SGK1 is different from that of PKB/Akt, as SGK1 does not contain a PH or any similar phosphoinositide-binding domain. SGK1 becomes phosphorylated on Ser422 in the C-terminal region termed the hydrophobic motif (H-motif) by a so-far unknown kinase that is PI-3K-dependent. This kinase activity has been referred to as PDK-2 or H-motif kinase activity; its activity can be indirectly inhibited by PI-3K inhibitors such as Wortmannin or LY-294002 (Kobayashi and Cohen 1999; Park et al., 1999) or mimicked by mutation of Ser422 to aspartate, thereby generating a constitutively active kinase. Phosphorylation of Ser422 or mutation to aspartate transforms SGK1 into a substrate for PDK-1, which binds via its PDK- 1-interacting fragment (PIF)–binding pocket to the H-motif on SGK1, promoting phosphorylation of the A-loop motif and consequently activation of SGK1 (Biondi et al., 2001). Hence, in this context, and in contrast to PKB/Akt activation, phosphorylation of SGK1 by PDK-1 is independent of phosphoinositides or the PH domain in PDK-1. This renders the activation of SGK1 much slower (with maximal activation occurring typically after 10 to 40 minutes) than that of PKB/Akt. The physiological significance of this difference is unclear but suggests a bigger need for rapid activation of PKB/Akt than of SGK1. Changes in Na^+ balance (whereby SGK1 is activated via aldosterone) usually occur less rapidly than changes in blood glucose levels (whereby PKB/Akt is activated). cAMP also may activate SGK1 (Perrotti et al., 2001). In this context, a consensus sequence for PKA-dependent phosphorylation surrounding Thr369 (K-K-I-T-P), which is necessary for cAMP-dependent phosphorylation, was identified. However, this consensus for phosphorylation is imperfect, and other groups have been unable to detect activation of SGK1 by cAMP under conditions in which cAMP stimulated the phosphorylation of the transcription factor CRE phosphorylated and activated by various MAP kinases, namely by bone marrow kinase (BMK) [also known as extracellular signal–regulated kinase 5 (ERK5)] or by p38α

(Hayashi et al., 2001; Meng et al., 2005). Both these kinases phosphorylate SGK1 on Ser78, which lies outside of the catalytic domain. Such phosphorylation leads to an increase in Sgk1 activity, independent of the phosphorylation state of Thr256 (in the A-loop). It is not known how this phosphorylation activates SGK1, but other kinases are also activated by phosphorylation outside of the catalytic domain (Stokoe et al ., 1992). SGK1 has also been reported to interact with WNK1 (*w*ith *n*o *lysine k*inase *1*), a kinase playing a role in Na^+ homeostasis and blood pressure control. In this process WNK1 is able to activate SGK1. The mechanism of activation is not completely clear, but it may involve phosphorylation of Ser422 or mutation of Ser422 to aspartate in addition to phosphorylation of Thr256, suggesting a similar mechanism as that involved in the activation via PDK-1. However, direct phosphorylation of SGK1 by WNK1 has not been demonstrated (Xu et al., 2005).

1.4 Tissue Distribution of SGK Isoforms

SGK isoforms are expressed in numerous tissues and cell lines. Among the three kinases, SGK1 (Webster et al., 1993; Waldegger et al., 1997; Kobayashi et al., 1999) and Sgk3 (Kobayashi et al., 1999) show the broadest distribution, with expression in many tissues including the brain, placenta, lung, liver, pancreas, kidney, heart and skeletal muscle. In situ hybridization studies localized SGK1 mRNA in several epithelial and/or nonepithelial cells within the brain (Warntges et al., 2002; Nishida et al., 2004; Tsai et al., 2002; Stichel et al., 2005; Gonzalez-Nicolini and McGinty 2003), eye (Rauz et al., 2003; Rauz et al., 2003a), lung (Waerntges et al., 2002), liver (Fillon et al., 2002), ovary (Alliston et al., 2000), pancreas (Klingel et al., 2000), intestine (Waldegger et al., 1999), and kidney (Chen et al., 1999; Friedrich et al., 2002; Huber et al., 2001). SGK1 mRNA expression is established very early in embryonic development, as indicated by in situ hybridizations on whole-mount preparations of mouse embryo (Lee et al., 2001). By embryonic day (E) 8.5, SGK1 is already highly expressed in the decidua and yolk sac. By days E9.5– E12.5 it is found in the developing heart, eye, and lung, and it becomes highly expressed by days E13.5–E16.5 in the brain choroid plexus, kidney distal tubules, bronchi/brochioli, adrenal glands, liver, thymus, and intestine (Lee et al., 2001). In contrast to SGK1 and SGK3, SGK2 reveals a more restricted distribution and is highly abundant only in the liver, kidney, and pancreas, where it is found in two different SGK2 species, referred to as SGK2α and

SGK2β (Kobayashi et al., 1999). SGK isoform expression varies also between cell lines cultured *in vitro*. Similar to its expression pattern *in vivo*, SGK1 is broadly expressed in cultured cells and is readily detectable in, for example, hepatoma cells, fibroblasts, and mammary tumor cells (Webster et al., 1993; Kobayashi et al., 1999). By contrast, SGK2 mRNA is expressed in hepatoma cells but not in fibroblasts, whereas SGK3 is found in fibroblasts but not in hepatoma cells. Remarkably, all three SGK isoforms are expressed in cells derived from the renal cortical collecting duct (Naray-Fejes-Toth et al., 2004).

1.5 Role of SGK1 in Aldosterone Dependent Na^+ Reabsorption

Although SGK isoforms are expressed in various tissues and cell types, the role of SGK1 in aldosterone-dependent regulation of Na^+ homeostasis is the best-studied function of these kinases with respect to epithelial ion transport. The kidneys play a pivotal role in the maintenance of Na^+ homeostasis. Urinary Na^+ excretion must be tightly regulated to maintain a constant extracellular volume during varying dietary Na^+ intake and extrarenal Na^+ losses. The final control of renal Na^+ excretion is achieved by the ASDN i.e. the late distal convoluted tubule, the connecting tubule, and the cortical as well as the medullary collecting ducts (CCD and MCD respectively) (Loffing et al., 2001). Transepithelial Na^+ transport in these segments is accomplished by Na^+ entry into the epithelial cells via the epithelial Na^+ channel (ENaC) in the luminal membrane and by exit of Na^+ through the Na^+, K^+-ATPase in the basolateral plasma membrane. ENaC represents the rate limiting step in this process and is highly regulated (Kellenberger and Schild 2002). It is composed of three subunits (α, β and γ) (Canessa et al., 1994; Canessa et al., 1994; Lingueglia et al., 1993; Lingueglia et al., 1994) with a stoichiometry of $2\alpha1\beta1\gamma$ (Firsov et al., 1998), although other stoichiometries have also been proposed (octa- or nonamers) (Eskandari et al., 1999; Snyder et al., 1998). Its subunits have a similar topology, with two transmembrane domains, one extracellular loop, and two cytoplasmic ends (Renard et al., 1994; Canessa et al., 1994; Snyder et al., 1994). Each subunit also contains, at its C-terminal end, a PY-motif (P-P-X-Y, where P is a proline, Y a tyrosine, and X any amino acid), which is known as protein:protein interaction motifs that can interact with tryptophan (W)-rich WW domains (Chen and Sudol 1995; Staub and Rotin 1996). The importance of these PY-motifs for ENaC regulation has been recognized by the findings that most cases of Liddle's syndrome

(Liddle et al., 1963), an inherited form of salt-sensitive hypertension are caused by mutations in the genes encoding β- and γ -ENaC (Hansson et al., 1995; Shimkets et al., 1994). These mutations invariably cause either the deletion or the mutation of the PY-motifs on these subunits. When such Liddle channels are expressed in heterologous systems, increases in both the density at the cell surface and the open probability of ENaC are observed (Firsov et al., 1996; Snyder et al., 1995; Schild et al., 1995; Schild et al., 1996). Loffing and his coworkers, has demonstrated that these PY-motifs are the binding sites for ubiquitin-protein ligases of the Nedd4/Nedd4-like family (Kamynina et al., 2001; Kamynina et al., 2001a) and particularly of Nedd4-2 (Kamynina et al., 2001; Kamynina et al., 2001a; Snyder et al., 2004). It is thought that Nedd4-2 binds via its WW domains with the PY-motifs of ENaC and ubiquitylates ENaC on its α and γ subunits, consequently leading to the internalization and degradation of ENaC in the endosomal/lysosomal system (Snyder et al., 2004). In Liddle's syndrome, this mechanism is impaired owing to the inactivation of a PY-motif, causing the accumulation of ENaC at the plasma membrane (Kamynina and Staub 2002). The activity of ENaC and the Na^+, K^+-ATPase is tightly regulated by aldosterone and by SGK1 (Kellenberger and Schild 2002; Vallon et al., 2005; Bhargava et al., 2004). Experiments in heterologous expression systems (i.e., *X. laevis* oocytes) revealed that coexpression of either ENaC or Na^+,K^+-ATPase (Zecevic et al., 2004; Setiawan et al., 2002) with SGK1 profoundly increases the activity of both Na^+-transporting proteins. Likewise, SGK2 and SGK3 stimulate ENaC (Friedrich et al., 2003) and Na^+,K^+-ATPase (Henke et al., 2002). The stimulatory effect of SGK1 on ENaC is related both to an increased number of channels in the plasma membrane (Lang et al., 2000; Loffing et al., 2001; Alvarez de la Rosa et al., 1999) and an activation of channels already present in the membrane (Diakov and Korbmacher 2004). The first effect likely involves the action of Nedd4-2, as there are several consensus phosphorylation motifs (2–3 depending on the splice variant) on Nedd4-2 and a PY-motif on SGK1 that may serve as a binding site for Nedd4-2. In *Xenopus* oocytes, SGK1 induces Nedd4-2 phosphorylation on two of these phosphorylation sites (primarily Ser444, but also Ser338) (Embark et al., 2004; Palmada et al., 2004; Debonneville et al., 2001), which decreases the interaction of Nedd4-2 with ENaC and finally leads to an enhanced expression and activity of ENaC at the cell surface (Debonneville et al., 2001). This inhibitory effect of SGK1 on Nedd4-2 likely involves 14-3-3 proteins as phosphorylation of Ser444 in Nedd4-2 creates a possible binding site for such proteins

(consensus: R-S-X-pS-X-P). Indeed, in *X. laevis* oocytes, SGK1 increases the binding of 14-3-3 to Nedd4-2 in a phosphorylation-dependent manner, a dominant-negative 14-3-3 mutant profoundly attenuates SGK1-dependent stimulation of ENaC, and overexpression of the 14-3-3 protein impairs Nedd4-2-dependent ubiquitylation of ENaC (Ichimura et al., 2005).

In addition to this indirect action of SGK1 on ENaC cell surface abundance, it was proposed that SGK1 can directly interact with ENaC (Wang et al., 2001) and increase ENaC channel activity by phosphorylating the α-ENaC subunits (Diakov and Korbmacher 2004). Diakov & Korbmacher (2004) used outside-out membrane patches of *X. laevis* oocytes expressing rat ENaC to demonstrate that addition of recombinant, constitutively active SGK1 directly stimulates ENaC currents two- to threefold. An alanine mutation of the serine residue in the SGK1 consensus R-X-R-X-X-S phosphorylation motif abolishes the stimulatory effect on ENaC in this experimental setting. Experiments in native *Xenopus* A6 cells expressing endogenous SGK1 and ENaC further confirmed that the action of SGK1 on ENaC is complex and likely involves (*a*) increases in the subunit abundance in the plasma membrane and (*b*) activation of channels already in the plasma membrane combined with an increase in ENaC open probability (Alvarez de la Rosa et al., 2004). However, in this model the stimulatory effect on ENaC channel activity cannot be explained by a direct SGK1-dependent phosphorylation of α-ENaC because *Xenopus* α-ENaC does not contain the SGK1 consensus phosphorylation motif. That direct phosphorylation of ENaC at the SGK1 consensus site is not essential for ENaC activation is also supported by data from Lang and coworkers (Lang et al., 2000; Friedrich et al., 2002) that showed that channels with a serine-to-alanine mutation within the consensus site of α-ENaC are still rigorously upregulated by coexpression of SGK1 in *Xenopus* oocytes. NDRG-2, which is an aldosterone-induced protein in the ASDN, is another target of SGK1 (Boulkroum et al., 2002; Murray et al., 2004). Although the functional role of NDRG-2 in the ASDN is not known, this protein may also have some function in the SGK1-dependent signaling cascade related to Na^+ transport. As an aldosterone-induced protein, SGK1 is thought to mediate at least some of the physiological effects of aldosterone on ENaC and Na^+,K^+-ATPase. The stimulatory effect of aldosterone (or of dexamethasone) on SGK1 expression has now been firmly documented in several studies on various *in vitro* and *in vivo* systems, including *Xenopus* A6 cells (Bhargava et al., 20004), primary rabbit CCD cells (Narey-Fejes-Toth et

al., 1999), mouse inner MCD cells (Gumz et al., 2003), mouse mpkCCDcl4 (Flores et al., 2005), mouse M1 cells (Helms et al., 2003), and mouse and rat kidneys (Chen et al., 1999; Loffing et al., 2001; Bhargava et al., 2001). Corticosteroids rapidly (within 30 minutes) induce SGK1 at the mRNA and/or protein levels. This induction precedes or at least coincides with enhanced phosphorylation of Nedd4-2 (Flores et al., 2005), the activation of transepithelial Na^+ transport in cultured renal epithelia (Naray-Fejes-Toth et al., 1999; Bhargava et al., 2004; Flores et al., 2005), and reduced renal Na^+ secretion in intact animals (Bhargava et al., 2001). At least part of the stimulatory effect of aldosterone on SGK1 appears to be mediated by activation of the MR, as indicated by findings in primary rabbit collecting duct cells *in vitro* (Naray-Fejes-Toth et al., 1999) and kidneys *in vivo* (Bhargava et al., 2001). Consistently, physiologically relevant concentrations of aldosterone are sufficient to significantly induce SGK1 mRNA in the renal cortex and outer medulla (Muller et al., 2003). The physiological importance of aldosterone in SGK induction is also supported by the fact that dietary Na^+ restriction, which physiologically increases plasma aldosterone, induces SGK1 mRNA in the renal cortex (Farjah et al., 2003). The aldosterone-dependent induction of SGK1 occurs specifically in the ENaC-positive cells of the ASDN, whereas SGK1 expression in other nephron portions such as the thick ascending limb or the proximal tubule is not increased by aldosterone. Likewise, the high level of expression of SGK1 in the renal papilla is not further stimulated by aldosterone, suggesting that SGK1 expression at this site is controlled by factors other than aldosterone. The renal papilla plays an important role for the urinary concentration mechanism, and the cells in the renal papilla can be exposed to a large variation in extracellular osmolarity depending on the requirements for diuresis to antidiuresis. SGK1 expression is strongly modulated by osmotic cell shrinkage and swelling (Waldegger et al., 1997; Rozansky et al., 2002), and it is therefore conceivable that SGK1 participates in the functional adaptation of the renal papilla cells to fluctuation of extracellular osmolarity. Consistent with this notion, recent data suggest that SGK1 mediates the osmotic induction of the type A natriuretic peptide receptor (NPR-A) in rat inner MCD cells (Chen et al., 2004). Aldosterone also controls SGK1 expression in the distal colon (Coric et al., 2004; Bhargava et al., 2001). Aldosterone-dependent Na^+ reabsorption at this site may help to limit extrarenal Na^+ losses during conditions of dietary Na^+ restriction. Transepithelial Na^+ transport is achieved mainly by epithelial cells that are situated at the tips of colonic crypts and that express high levels of

ENaC (Coric et al., 2004) and Sgk1 (Waldegger et al., 1999; Coric et al., 2004). In spite of these data pointing to aldosterone-dependent regulation of ENaC via SGK1, recent Western blot and immunohistochemical studies on rat kidney and colon, which reported no or rather modest aldosterone-dependent induction of SGK1 at the protein level, were interpreted to question the significance of aldosterone-dependent induction of SGK1 for ENaC-mediated Na^+ transport regulation (Coric et al., 2004). Support for a functional significance of SGK1 in regulation of transepithelial Na^+ transport comes from experiments in *X. laevis* A6 cells and in mouse M1 CCD cells. Transfection of A6 or M1 cells with SGK1 leads to an increase in transepithelial Na^+ transport, whereas transfection of a dominant-negative "kinase-dead" SGK1 mutant or an antisense SGK1 transcript abolishes dexamethasone- and/or insulin-dependent regulation of transepithelial Na^+ transport (Alvarez de Rosa et al., 2003; Faletti et al., 2002). Likewise, the use of interfering RNA to knockdown SGK1 expression in A6 cells results in a significant reduction in SGK1 protein levels and a ~50% reduction in dexamethasone-induced short-circuit currents (Bhargava et al., 2004). Consistent with these *in vitro* findings, experiments in SGK1 KO ($sgk1^{-/-}$) mice supported the importance of SGK1 for aldosterone-dependent regulation of renal Na^+ transport (Wulff et al., 2002). Under a standard diet, the KO mice have unaltered Na^+ excretion as compared to their wildtype littermates. However, plasma aldosterone levels are significantly increased in $sgk1^{-/-}$ mice, suggesting extracellular volume contraction. Under dietary Na^+ restriction, activated compensatory mechanisms are no longer sufficient to keep the mice in Na^+ balance, and mice disclosed significant loss in renal NaCl and in body weight. Experiments on collecting ducts perfused ex vivo revealed significantly lower transepithelial amiloride-sensitive potential differences, consistent with a reduced Na^+ transport activity in the CCD. Although apical localization of ENaC was seen in both Na^+-restricted $sgk^{+/+}$ and $sgk1^{-/-}$ mice, the apical localization of ENaC is inappropriately low in the $sgk1^{-/-}$ mice given the severalfold higher plasma aldosterone levels in the KO mice. Nevertheless, these data, together with the rather mild phenotype of $sgk1^{-/-}$ mice, as compared to the much more severe and life-threatening phenotypes of MR or ENaC KO mice, suggest that (*a*) aldosterone-dependent control of ENaC function does not solely rely on the induction and activation of SGK1 and (*b*) some redundancy exists in the signal transduction pathway that controls ENaC activity. Consistent with these ideas, Loffing and his coworkers found significant phosphorylation of the SGK1 target Nedd4-2 in mouse mpkCCDcl4 cells *in vitro*

and in rat collecting ducts *in vivo* in the absence of any aldosterone and detectable SGK1 protein expression (Flores et al., 2005). In addition to aldosterone-dependent regulation of renal Na^+ reabsorption, SGK1 appears to be involved also in the regulation of aldosterone-induced salt appetite. *Sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice show a similar salt intake under standard conditions. Treatment with the synthetic aldosterone analogue deoxycorticosterone-acetate (DOCA) increases Na^+ intake much more in *Sgk1*$^{+/+}$ mice than in *sgk1*$^{-/-}$ mice. The underlying mechanism for the reduced mineralocorticoid-induced salt intake is unclear (Vallon et al., 2005).

1.6 Role of SGK1 in Renal K^+ Secretion

Aside from its stimulatory effect on renal Na^+ reabsorption, aldosterone has strong kaliuretic action. Renal K^+ secretion also takes place in the ASDN and is likely mediated by the renal outer medullary K^+ channel ROMK. ROMK is coexpressed with ENaC in the ASDN cells, and Na^+ reabsorption via ENaC provides the necessary driving force for K^+ secretion. Consistently, pharmacological inhibition (i.e., by amiloride) or genetic loss of function [i.e., pseudohypoaldosteronism (PHA) type 1] of ENaC lower renal K^+ secretion and predispose one to hyperkalemia. It remains unresolved whether the kaliuretic effect of aldosterone is entirely secondary to the activation of ENaC-mediated Na^+ reabsorption or whether aldosterone directly regulates ROMK function. Patch-clamp studies on rat CCDs found no measurable effect of acute aldosterone administration on K^+ channel number, open probability, or conductance (Palmer et al., 1994). However, some data suggested that aldosterone induces renal K^+ secretion already at aldosterone concentrations that do not exhibit any measurable effect on urinary Na^+ excretion (Bhargava et al., 2001). Moreover, high K^+ intake increases ROMK activity more efficiently in intact rats than in adrenalectomized animals, suggesting that aldosterone may have at least a permissive effect on ROMK activation (Palmer et al., 1994). Consistent with a possible role of aldosterone in ROMK regulation, recent studies in heterologous expression systems advocated a regulatory action of aldosterone-induced SGK1 on ROMK cell surface activity and abundance (Palmada et al., 2003, Palmada et al., 2003a). The regulatory role of SGK1 with regard to ROMK may be indirect via increased interaction with the Na^+, H^+ exchanger–regulating factor 2 (NHERF2) (Palmada et al., 2003, Palmada et al., 2003a) or direct via

increased phosphorylation of ROMK at a serine residue within the canonical SGK1 consensus phosphorylation motif (Yoo et al., 2003). The *in vivo* significance of SGK1 in regulation of renal K^+ transport was recently analyzed in SGK1 KO mice. These mice indeed show a disturbed adaptation to an acute and chronic K^+ load, but, as indicated by electrophysiological and immunohistochemical data obtained from these mice after a chronic potassium load, this maladaptation likely is related to altered ENaC (or Na^+,K^+-ATPase) activity in the ASDN cells rather than to inhibition of ROMK cell surface targeting or activity (Huang et al., 2004).

1.7 Serum and glucocorticoid-inducible kinase 1 and blood pressure

A potential role for impaired regulation of SGK1 in NaCl-sensitive hypertension was suggested from studies in Dahl rats: whereas a high NaCl diet lowered renal expression of SGK1 and did not affect blood pressure in Sprague–Dawley rats, the NaCl-sensitive Dahl rats showed an increased renal expression of SGK1 associated with an increase in blood pressure (Farjah et al., 2003). Unfortunately, the nephron site of enhanced SGK1 expression was not determined. As outlined above, activation as well as gain-of-function mutations of SGK1 could increase renal Na^+ reabsorption and, thus potentially, blood pressure through affecting various transport systems including (1) NHE3 and KCNQ1/KCNE1 in proximal tubule, (2) BSC-1 in thick ascending limb, (3) ENaC in ASDN and (4) basolateral Na+/K+-ATPase in all nephron segments. Moreover, preliminary studies provided evidence that the increase in NaCl appetite in response to the mineralocorticoid deoxycorticosterone acetate is attenuated in mice lacking SGK1 (Vallon et al., 2005), indicating that SGK1 may play at least a dual role in mineralocorticoid-regulated NaCl homeostasis. SGK1- dependence of both NaCl intake and renal NaCl reabsorption would make the kinase an attractive candidate gene for arterial hypertension with gain-of function mutations stimulating NaCl intake in the presence of an impaired ability to excrete NaCl through the kidney. A study by Busjahn et al. (2002) indeed identified two polymorphisms of the SGK1 gene, one synonymous polymorphism in exon 8 (E8CC/CT) and one in intron 6 (I6CC) that correlate with enhanced blood pressure in twin studies. Individuals carrying the gene variants that associated with higher blood pressure also have an increased body mass index that may be due to stimulation of Na^+/glucose transporter SGLT1 in the intestine (Dieter et al., 2004).

Moreover, they have a tendency for shorter cardiac action potential possibly due to enhanced activation of KCNE1/KCNQ1 (Busjahn et al., 2004). In contrast, Trochen et al. (Trochen et al., 2004) recently studied the aforementioned polymorphisms in exon 8 and intron 6 in unrelated Caucasian healthy controls and essential hypertensive patients, and concluded that these polymorphisms do not have a significant effect on blood pressure in subjects without renal disease, do not account for the occurrence of renal disease per se and are not determinants of progressive renal failure. The different results with regard to blood pressure could be due to relatively small sample size and the fact that the effect of the gene variant in twins has been small (Busjahn et al., 2002) and may not be apparent in selected populations, illustrating the problems in determining genetic influences in complex traits. In any case, additional effort is needed to elucidate the impact of SGK1 on blood pressure control and genesis of hypertension.

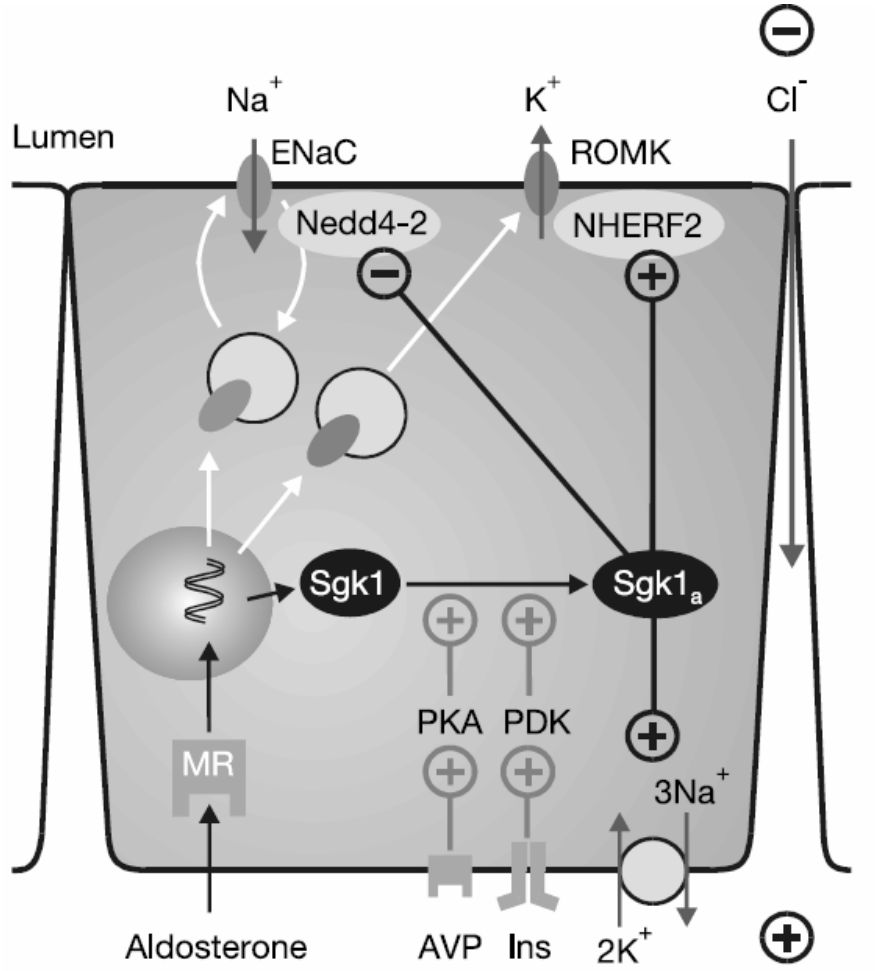

Figure 1. Proposed interaction between SGK1, epithelial Na^+ channel (ENaC), and renal outer medullary K^+ channel (ROMK) in aldosterone-sensitive distal nephron. Aldosterone stimulates the expression of ENaC, ROMK and SGK1. Activation of SGK1 (Sgk1a) requires phosphorylation of the kinase, which, in addition to other factors, can be induced by binding of insulin (Ins) or arginine vasopressin (AVP) to their basolateral receptors. Sgk1 is not absolutely required for insertion of ENaC or ROMK into the apical membrane, explaining the mild phenotype of SGK1-deficient mice under standard NaCl and K^+ diet. SGK1-dependent upregulation of Na^+ reabsorption, however, is required under reduced dietary NaCl intake as well as for upregulation of renal K^+ excretion in response to increased dietary K^+ intake. SGK1a increases Na^+ reabsorption by activating Na^+-K^+-ATPase and enhancing the abundance in the cell membrane of ENaC through inhibition of ubiquitin ligase Nedd4–2 mediated internalization of ENaC. Effects of SGK1a on ENaC and Na^+-K^+-ATPase increase the electrical driving force for paracellular Cl^+ reabsorption as well as the electrochemical driving force for K^+ secretion through ROMK. In addition,

SGK1 may enhance the abundance of ROMK in the apical membrane by synergizing with NHERF2. MR, mineralocorticoid receptor; PKA, protein kinase A; PDK, 3-phosphoinositide-dependent kinase.

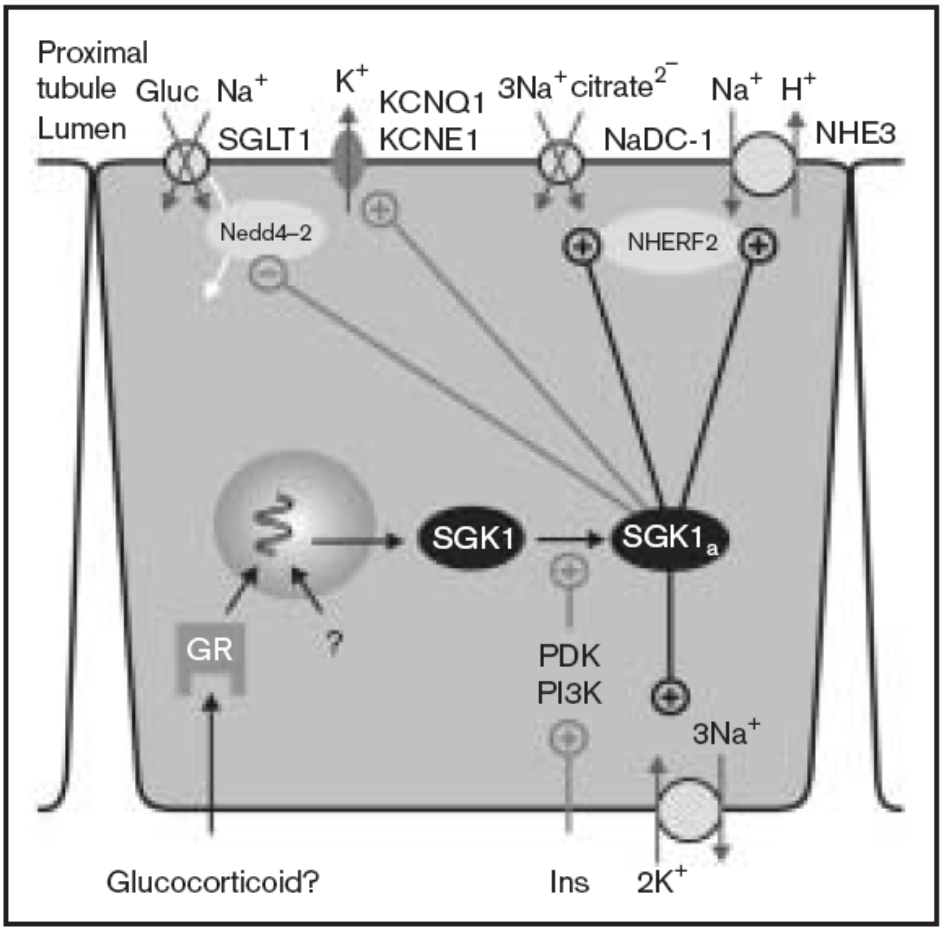

Figure 2: (1) Acute hyperglycemia increases plasma insulin (Ins) concentration, which activates SGK1 (SGK1a). Activated SGK1 stimulates the electrogenic Na^+/glucose cotransporter SGLT1 by inhibiting Nedd4-2- mediated retrieval of SGLT1 from the cell membrane, and to prevent membrane depolarization SGK1 co-activates the potassium channel KCNQ1/KCNE1. This enhances the glucose reabsorption capacity of proximal tubule to prevent postprandial glucosuria. (2) SGK1 activates both Na^+/H^+ exchanger NHE3 and electrogenic Na+/dicarboxylate transporter NaDC-1 by a mechanism involving NHE-regulating factor 2 (NHERF2). Under conditions of metabolic acidosis, SGK1 may serve to coordinate proton secretion through NHE3 and citrate reabsorption through NaDC-1 with citrate being used for bicarbonate generation. Stimulation of basolateral Na^+/K^+-ATPase by SGK1 increases the electrochemical gradient for Na^+ reabsorption across the luminal membrane. The factors that control the expression of SGK1 in proximal tubule remain to be determined. GR, glucocorticoid receptor; PI3K, phosphatidylinositol 3-kinase; PDK, 3-phosphoinositidedependent kinase.

II. AIMS OF THE STUDIES

The aim of the present work was to answer the following questions:

Study 1: What is the influence of SGK1 on renal function and systolic blood pressure during high fat diet and high salt diet?

Study 2: What is the role of SGK1 in the hypertensive effect of combined treatment with fructose and salt load?

Study 3: What is the role of SGK1 in the regulation of electrolyte metabolism and blood pressure by the glucocorticoid dexamethasone?

Study 4: What is the role of SGK1 in salt sensitivity of glucose tolerance and peripheral glucose uptake?

II. 1. <u>Study 1:</u> Influence of SGK1 on renal function and systolic blood pressure during high fat diet and high salt diet

Expression of the serum and glucocorticoid inducible kinase SGK1 (Firestone et al., 2003) is strongly upregulated by mineralocorticoids (Bhargava et al., 2001; Brennan and Fuller 2000; Chen et al., 1999; Djelidi et al., 2001; Loffing et al., 2001; Narey-Fejes-Toth et al., 1999; Pearce 2003; Shigaev et al., 2000; Verrey et al., 2003). For activation, SGK1 requires phosphorylation which is accomplished by the phosphoinositide dependent kinase PDK1 (Biondi et al., 2001; Kobayashi and Cohen 1999; Park et al., 1999). Stimulators of SGK1 activity include insulin which activates SGK1 through a signalling pathway involving phosphatidylinositide 3 kinase and PDK1 (Kobayashi and Cohen 1999).

SGK1 interacts with the epithelial Na^+ channel ENaC (Wang et al., 2001) and coexpression of SGK1 markedly enhances the activity of ENaC heterologously expressed in *Xenopus* oocytes (Alvarez de la Rosa et al., 1999; Bohmer et al., 2000; Chen et al., 1999; Lang et al., 2000; Narey-Fejes-Toth et al., 1999; Wagner et al., 2001) and in A6 cells (Alvarez et al., 2004; Faletti et al., 2002; Rozansky et al., 2002; Verrey et al., 2003). SGK1 increases ENaC in part by phosphorylation of the ubiquitin ligase Nedd4-2 (Debonneville et al., 2001; Snyder et al., 2002) which ubiquitinates ENaC thus preparing the channel protein for clearance from the cell membrane and subsequent degradation (Staub et al., 1997). Phosphorylation of Nedd4-2 by SGK1 reduces the affinity of the enzyme to the target proteins and thus disrupts the ubiquitination of ENaC leading to enhanced ENaC channel protein abundance in the cell membrane (Alvarez de la Rosa et al., 1999; Lang et al., 2000; Wagner et al., 2001). Beyond that SGK1 may regulate ENaC activity by more direct interaction (Diakov et al., 2004)

SGK1 is expressed in the aldosterone sensitive distal nephron (Alvarez and Canessa 2003; Loffing et al., 2001) and the stimulation of ENaC by SGK1 is considered to participate in the regulation of renal Na^+ excretion by aldosterone, insulin and IGF-1 (Blazer-Yost et al., 1998; Blazer-Yost et al., 1999; Blazer-Yost et al., 2004; Wang et al., 2001). Besides ENaC, SGK1 enhances the activity of further renal transport systems, including the apical K^+ channel ROMK (Palmada et al., 2003; Yun et al., 2002), the Na^+/K^+-ATPase (Henke et al., 2002; Setiawan et al., 2002; Zecevic et al., 2004), the Na^+, K^+, $2Cl^-$ cotransporter NKCC2 (Lang et al., 2000), the epithelial Ca^{2+} channel TRPV5 (Embark et al., 2004; Palmada et al.,

2005), the Na^+/H^+ exchanger NHE3 (Yun 2003; Yun et al., 2002) and the K^+ channel KCNE1 (Embark et al., 2003).

Considering the significance of renal Na^+ transport for blood pressure regulation (Lifton 1996; Luft 2004), the effects of SGK1 on ENaC were expected to influence blood pressure. As a matter of fact, moderately enhanced blood pressure is observed in individuals carrying a variant of the SGK1 gene affecting as many as 5% of unselected Caucasians (Busjahn et al., 2002; Busjahn and Luft 2003). Most recent evidence disclosed a relatively strong correlation between insulinemia and blood pressure in individuals carrying this SGK1 gene variant suggesting a particular role of SGK1 in the hypertension paralleling hyperinsulinemia (von Wowern et al., 2005).

Gene targeted mice lacking SGK1 (*sgk1*$^{-/-}$) show normal Na^+ excretion and blood pressure under normal salt intake, but their ability to retain Na^+ and maintain blood pressure under a salt deficient diet is impaired (Wulff et al., 2002). In contrast to the mild phenotype of the *sgk1*$^{-/-}$ mouse, targeted disruption of the mineralocorticoid receptor in mice leads to severe salt wasting (Berger et al., 1998) and the ENaC knockout mouse is not viable (Hummler et al., 1996). Thus, SGK1 is not required for the function of ENaC but significantly contributes to, yet does not fully account for, the effects of mineralocorticoids in the kidney. The role of SGK1 in preventing a decrease of blood pressure following salt depletion does not necessarily imply that the lack of SGK1 protects from an increase of blood pressure following excessive salt intake which was expected to decrease plasma aldosterone concentrations and thus to decrease SGK1 expression in wild type mice. Thus, high salt diet could dissipate the difference between *sgk1*$^{-/-}$ mice and wild type mice. Thus, the question arises whether and under which conditions SGK1 may be relevant for blood pressure increase.

The present study has been performed to explore the influence of SGK1 on renal function and systolic blood pressure during high fat diet and high salt diet, which have both been shown to favour increase of blood pressure (Boustany et al., 2004; Cook et al., 2004; Cubeddu et al., 2000; Dobrian et al., 2003; Grant et al., 2002; Jang et al., 2004; Laffer et al., 2004; Ogihara et al., 2002; Rocchini et al., 2004).

[The studies referring to these aims, which were displayed in the further sections, have been published as an original publication (Huang and Boini et al., 2006a)]

II. 2. <u>Study 2:</u> Role of SGK1 in the hypertensive effect of combined treatment with dietary fructose and salt load

Dietary fructose leads to hyperglycemia, hyperinsulinism and increase in blood pressure (Catena et al., 2003; Hsieh 2004; Nandhini and Anuradha 2004; Shinozaki et al., 2004; Song et al., 2004; Zhao et al., 2003). Since 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 (DeFronzo et al., 1976; Gupta et al., 1992; Muscelli et al., 1996). The antinatriuretic effect of insulin appears to result from direct action on tubular transport in distinct nephron segments (Catena et al., 2003; El Atat et al., 2004; Hayashida et al., 2001; Song et al., 2004). Insulin stimulates ENaC and may thus lead to renal retention of NaCl (Bickel et al., 2001; Song et al., 2004; Zhang et al., 2005), which in turn favours the development of hypertension (Lifton 1996; Luft 2004). The effect of insulin on ENaC requires activation of the Phosphatidylinositide-3 (PI3)- kinase (Blazer-Yost et al., 2003; Blazer-Yost et al., 2004; Tong et al., 2004). The regulation of ENaC activity by insulin may involve the serum and glucocorticoid inducible kinase SGK1 (Firestone et al., 2003). Expression of SGK1 has previously been shown to be stimulated by mineralocorticoids (Bhargava et al., 2001; Brennan and Fuller 2000; Chen et al., 1999; Djelidi et al., 2001; Loffing et al., 2001; Narey-Fejes-Toth et al., 1999; Pearce 2003; Shigaev et al., 2000; Verrey et al., 2003)**.** SGK1 is activated by insulin through a signalling cascade involving PI-3 kinase and PDK1 (Biondi et al., 2001; Kobayashi et al., 1999; Park et al., 1999). Targets of SGK1 action include the epithelial Na^+ channel ENaC (Alvarez de la Rosa et al., 1999; Böhmer et al., 2000; Chen et al., 1999; Lang et al., 2000; Narey-Fejes-Toth et al., 1999; Wagner et al., 2001).

Chronic fructose feeding causes arterial hypertension which is associated with insulin resistance and hyperinsulinemia in rats (Catena et al., 2003; Hwang et al., 1987; Verma et al., 1994). The present study aimed to explore the role of SGK1 in the hypertensive effect of combined treatment with 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.

[The studies referring to these aims, which were displayed in the further sections, have been published as an original publication (Huang and Boini et al., 2006b)]

II. 3. Study 3: Role of SGK1 in the regulation of electrolyte metabolism and blood pressure by the glucocorticoid dexamethasone

The serum and glucocorticoid inducible kinase SGK1 has originally been cloned as glucocorticoid sensitive gene (Firestone et al., 2003) and later has been shown to be similarly sensitive to genomic regulation by mineralocorticoids (Bhargava et al., 2001; Brennan and Fuller 2000; Chen et al., 1999; Djelidi et al., 2001; Loffing et al., 2001; Narey-Fejes-Toth et al., 1999; Pearce 2003; Shigaev et al., 2000; Verrey et al., 2003). SGK1 is activated by insulin (Kobayashi and Cohen 1999) through a signalling cascade involving PI-3 kinase and PDK1 (Biondi et al., 2001; Kobayashi and Cohen 1999; Park et al., 1999).

Nothing is known about the contribution of SGK1 to the effects of glucocorticoids on renal function. Previously, glucocorticoids have been reported to increase blood pressure (Ferrari et al., 2002; Kenyon et al., 1990; Sacerdote et al., 2005), enhance renal tubular sodium transport (Bonvalet 1998), or increase fractional renal calcium excretion (Boross et al., 1986; Ferrari et al., 2002; Suzuki et al., 1983). They have been shown to decrease (Ferrari et al., 2002) or increase (Akeno et al., 2000; Lee et al., 2006) plasma Ca^{2+} concentration. The increase of plasma concentration may result in part from demineralization of bone (Patschan et al., 2001) and the hypocalcemia from renal loss of Ca^{2+} (Ferrari et al., 2002).

The present study explored the role of SGK1 in the regulation of electrolyte metabolism and blood pressure by the glucocorticoid dexamethasone. To this end, experiments have been performed in *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ mice prior to and following dexamethasone treatment without and with additional saline load.

[The studies referring to these aims, which were displayed in the further sections, have been submitted for publication (Boini et al., 2006c)]

II. 4. Study 4: Role of SGK1 in salt sensitivity of glucose tolerance and peripheral glucose uptake

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 (Cheng et al., 2001; Gaboury et al., 1994; Giner et al., 2001; ter Maaten et al., 1999; Vincent et al., 2003). Mechanisms accounting for the salt sensitivity of peripheral glucose uptake have remained ill defined. Glucose transport into insulin responsive cells is accomplished in large parts by insertion of the Na^+ independent glucose carriers GLUT1 (SLC2A1) and GLUT4 (SLC2A4) into the cell membrane (Cushman et al., 1980; James et al., 1988; Suzuki et al., 1980). The stimulation of the transporters by insulin requires phosphatidylinositide-3-kinase (PI3-kinase) and is abrogated by pharmacological inhibitors (Wortmannin and LY294002) or genetic knockout (PI3-kinase dominant-negative mutants) of the kinase (Bentley et al., 2003; Cheatham et al., 1994; Haruta et al., 1995; Kotani et al., 1995; Okada et al., 1994; Perrini et al., 2004). Downstream elements of PI-3-kinase include the phosphoinositide dependent kinase PDK-1, which in turn phosphorylates and thus activates the serine/threonine kinase Akt/protein kinase B (PKB) (Khan et al., 2002; Lang et al., 2001; Virkamaki et al., 1999). The effect of PI3-kinase on GLUT4 trafficking is mediated by PKB (Foran et al., 1999; Zhou et al., 2004). PKB is however, at least in some cells not required for the PI3-kinase dependent trafficking of GLUT1 (Foran et al., 1999). Thus, some other PI3-kinase dependent protein kinase is presumably involved in the regulation of GLUT1.

A further downstream molecule in the PI-3-kinase signalling cascade is the serum- and glucocorticoid- inducible kinase SGK1 (Biondi et al., 2001; Kobayashi et al., 1999; Park et al., 1999) which has previously been shown to stimulate several transport proteins (Lang et al., 2003) including the Na^+ coupled glucose transporter SGLT1 (Dieter et al., 2004). SGK1 was originally cloned as a glucocorticoid sensitive gene from rat mammary tumour cells (Firestone et al., 2003; Webster et al., 1993) and later as a human cell volume regulated gene (Waldegger et al., 1997). SGK1 is strongly upregulated by mineralocorticoids (Bhargava et al., 2001; Brennan and Fuller 2000; Chen et al., 1999; Djelidi et al., 2001; Loffing et al., 2001; Narey-Fejes-Toth et al., 1999; Pearce 2003; Shigaev et al., 2000; Verrey

et al., 2003) and participates in the stimulation of the renal epithelial Na^+ channel ENaC by mineralocorticoids (Chen et al., 1999; Pearce 2003; Shigaev et al., 2000; Verrey et al., 2003; Böhmer et al., 2000; Lang et al., 2000; Naray-Fejes-Toth et al., 1999) and insulin (Blazer-Yost et al., 2003; Blazer-Yost et al., 2004; Tong et al., 2004).

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.

[The studies referring to these aims, which were displayed in the further sections, have been published as an original publication (Boini et al., 2006d)]

III. METHODS

III. 1. Animals: 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 (Wulff et al., 2002; Huang et al., 2004). 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 ten generations and then intercrossed to generate homozygous SGK1 knockout mice (*sgk1*$^{-/-}$) and their wild type littermates (*sgk1*$^{+/+}$). In a first study, Male and female SGK1 knockout mice (*sgk1*$^{-/-}$) and their wild type littermates (*sgk1*$^{+/+}$) were fed either a control diet (1310/1314, 4 kcal% fat, 0.25% Na^+, 0.36% Cl^-, 0.71% K^+, Altromin, Heidenau, Germany) or a high fat diet (C1000, 45 kcal% fat, 0.25% Na^+, 0.36% Cl^-, 0.71% K^+, modified according D12451 from Research Diet, Altromin, Heidenau, Germany). Mice were maintained on standard diet until the age of 5 weeks when for half of the animals the diet was switched to high fat diet. Within the first 17 weeks mice were allowed free access to tap water. After 17 weeks, tap water was replaced by a 1% NaCl solution. In a second study, the C57BL/6J strain mice was used. This strain is commonly chosen to induce diabetes in mice and exhibits defects in glucose tolerance independent of obesity (Kaku et al., 1998: Kooptiwut et al., 2002). Male and female SGK1 knockout (*sgk1*$^{-/-}$) mice and their wild type littermates (*sgk1*$^{+/+}$) were fed on a control diet (1314, 0.2% Na^+, 0.4% Cl^-, 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 weeks. Consequently, the control diet was replaced by a high salt diet (1324, 4% NaCl, Altromin, Heidenau, Germany) and 10 % fructose was maintained as drinking solution for 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. In a third study, The 129/SvJ background mice were fed a control diet (1314, Altromin,

Heidenau, Germany) and had free access to tap drinking water. After a control period, the animals were treated with daily injections of dexamethasone (3 mg/kg b.w., i.p.) for 14 days. In a another series of experiments *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice were treated after a control period with daily injections of dexamethasone (3 mg/kg, b.w., i.p.) and 1% NaCl in drinking water for 14 days. In a fourth study, The genetic background of the 129/SvJ four months 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 used. The mice were fed a control diet (1310/1314, Altromin, Heidenau, Germany) and were allowed free access to tap water or to 1 % NaCl solution.

The following methods were used in all four studies where ever applicable.

III. 2. Glucose tolerance and insulin tolerance test: For determination of glucose tolerance, mice were starved overnight and glucose (3 g/kg body weight) was injected intraperitoneally (i.p.). 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 and 180 minutes after the injection. In another series of experiments, long acting insulin (Novo Nordisk, Mainz, Germany) was injected intraperitoneally (0.15U/kg, b.w.) and plasma glucose concentrations determined at 0, 15, 30, 45, 60 and 90 minutes as described above.

III. 3. Blood pressure: Systolic arterial blood pressure was determined by application of the tail-cuff method. As reviewed recently (Meneton et al., 2000), 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, semidarkend and clean environment, and performance of the measurements by one person and during a defined day time, when blood pressure is stable (between 1-3 PM). All these precautions were taken in the present study. The tail cuff method has the advantage to be noninvasive and can provide reproducible results of systolic blood pressure if those precautions are taken into account (Kurtz et al., 2005). Blood pressure recording was considered to be successful if the mouse did not move

and a clear initial pulse can be seen (Fig. 8). Five tail-cuff measurements were made in a session. The blood pressure for the session was accepted when the deviations of the five blood pressure readings were less than 5 mm Hg (Fig. 8).

III. 4 Metabolic cages: For evaluation of renal excretion, In a first study, both *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ mice were placed individually in metabolic cages (Tecniplast Hohenpeissenberg, Germany) for one week with free access to standard mouse diet or high fat diet both containing 0.25% Na^+, 0.36% Cl^- and 0.71% K^+ (Altromin, Heidenau, Germany). The inner wall of the metabolic cages was siliconized and urine was collected for 24 hours under water-saturated oil every second day when water and food intake were measured (Vallon 2003). Urinary excretion was determined again in metabolic cages as described above 25 days after exposure to 1% NaCl in drinking water under both, standard or high fat diet. In a second study, For evaluation of renal excretion, both, *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ mice were placed individually in metabolic cages (Techniplast Hohenpeissenberg, Germany) for 24 hour urine collection during a) control diet plus tap water (basal); b) 18 days on high salt diet plus tap water (high salt diet); c) 3 weeks on control diet plus 10% fructose in drinking water (fructose); d) 17 days on high salt diet plus 10% fructose in drinking water (fructose + high salt diet). In a third study, both, *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ mice were placed individually in metabolic cages (Techniplast Hohenpeissenberg, Germany) for 24 hour urine collection (Vallon 2003) during contorl diet, control diet plus dexamethasone treatment for 14 days and control diet plus dexamethasone treatment and 1% NaCl in drinking water for 14 days.

III. 5 Blood and urinary concentrations: To obtain blood specimen, animals were lightly anesthetized with isofluran (Abbott, Wiesbaden, Germany) and about 300 µl of blood was withdrawn into heparinized capillaries by puncturing the retro-orbital plexus. For determination of non-fasted blood glucose levels and plasma insulin concentrations, blood was drawn at 9 am in the morning and centrifuged immediately after the collection. Plasma and urinary concentrations of Na^+ and K^+ were measured by flame photometry (ELEX 6361, Eppendorf, Germany) and Cl^- concentrations were determined by electrometric titration (Chloridometer 6610, Eppendorf, Germany). Creatinine concentrations in urine and plasma was measured using an enzymatic kit (Demeditec, Kiel, Germany) Plasma concentrations of insulin were determined using an enzyme immunoassay kit (Mercodia,

Uppsala, Sweden), plasma aldosterone concentrations were measured using a commercial RIA kit (Beckman Coulter, Krefeld, Germany). Total cholesterol, triglycerides and free fatty acids were analysed using a colorimetric assay (BioVision Research; Sigma; Wako Chemicals). Phosphate and Ca^{2+} concentrations were determined colorimetrically utilizing commercial diagnostic kits (Roche Diagnostics, Mannheim, Germany).

III. 6 *In vivo* glucose uptake: *In vivo* tissue glucose uptake during a glucose tolerance test was determined in male mice that have been starved for 16 hours. 2-deoxy-D-[1,2-^{3}H]-glucose (2-DOG) was mixed with 20% of regular glucose (10 µCi/mouse, 3g/kg b.w.) and injected intraperitoneally. Blood glucose levels were determined to ensure adequate injection. After 120 minutes, the mice were killed and 100 mg of tissues were homogenized in 1 ml of water. 800 µl of 7% ice-cold perchloric acid was added to 800 µl of homogenate. The sample was then cleared by centrifugation and 1 ml of the supernatant was neutralized for 30 minutes with 2.2M $KHCO_3$. The precipitate was removed by centrifugation and 500 µl of the supernatant was used to determine total ^{3}H radioactivity.

III. 7 *In vitro* glucose uptake: To explore whether SGK1 increases glucose transport, HEK-293 cells were seeded on 6-well plates at 0.2 x 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, ^{3}H-deoxy-glucose (2-DOG) was used as the glucose analogue. 2-DOG uptake was measured in HEK-293 cells 2 days after transfection by incubating the cells at 37 °C for five minutes (linear range of uptake) in glucose-free Krebs Ringer HEPES (KRH) buffer containing 0.3 mM of unlabeled 2-DOG, 0.1 µCi/well [^{3}H] 2-DOG with or without 100 µM phloretin. Uptake was stopped by rapid aspiration of uptake solution, and washing four times with ice-cold PBS containing 50 mM unlabeled 2-DOG. Thereafter cells were lysed with 10 mM NaOH / 0.1 % Triton X-100 and radioactivity was measured with a liquid scintillation counter. Protein concentration was determined by the Bradford method.

III. 8 SGK1 protein abundance: In order to explore whether salt loading decreases SGK1 protein abundance, In one series of experiments, *sgk1*$^{+/+}$ mice were divided into 4 groups (5

mice per group) and treated separately with 1) Control diet for 3 months 2) Control diet for 3 months plus 1% NaCl in drinking water for 4 weeks 3) High fat diet for 3 months 4) High fat diet for 3 months plus 1% NaCl in drinking water for 4 weeks. In another series of experiments, *sgk1*$^{+/+}$ mice were divided into 4 groups (5 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 two weeks 3) Control diet for 2 weeks plus DOCA (35mg/kg b.w., s.c) treatment for two days 4) Control diet for 2 weeks plus DOCA (35mg/kg b.w., s.c.) and spironolactone (50mg/kg b.w., s.c) treatment for two days. Mice were anesthetized with ketamine (60mg/kg b.w., i.p.) plus xylazine (10mg/kg b.w., i.p.). Kidneys, skeletal muscle and fat tissues were removed and immediately shock frozen in liquid nitrogen. For Western blot analysis, the tissues were homogenised by using a homogeniser (Labortechnik, Mullheim, Germany). The kidneys were homogenised in lysis buffer containing 50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 50 mM sodium fluoride, 5 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 1% Triton X-100, 1% sodium deoxycholate, 1% sodium dodecyl sulphate and a protease inhibitor cocktail (Complete mini EDTA- free, Roche, Mannheim, Germany). The muscle tissues were homogenised in lysis buffer containing 50mM Tris-HCl, 50 mM NaF, pH 7, 50 mM β-glycerophosphate, 10 mM potassium phosphate, 10 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 1 mM sodium vanadate, 0.1% Tween 20 and a protease inhibitor cocktail tablet (Complete mini EDTA- free, Roche, Mannheim, Germany). The fat tissues were homogenised in lysis buffer containing 20 mM Tris, pH 7.4, 2 mM EDTA, 137 mM sodium chloride, 1% NP-40, 10% glycerol, 12 mM β-glycerol phosphate and a protease inhibitor cocktail tablet (Complete mini EDTA- free, Roche, Mannheim, Germany). The homogenates were centrifuged at approx. 7000 g, 4 °C for 15 min, the supernatant was removed and used for Western blotting. Total proteins were measured using the Bradford assay. Total proteins (100µg) were separated by SDS-page (10% Tris-Glycine), transferred to nitrocellulose membranes, blocked for overnight in blocking buffer (5% fat-free milk in PBS containing 0.1% Tween) at 4°C and incubated 1h with a polyclonal anti-SGK1 antibody (diluted 1:1000 in blocking buffer), kindly provided by Nicola Perrotti (Catanzaro, Italy), who generated the antibody by immunizing rabbits with the peptide EVLHQPYDRTVDW (SGK1 residues 267-280, Zymed laboratories, South San Francisco, CA) and subsequently characterized the specificity of the antibody (Menniti et al., 2005; Faletti et al., 2002). In single experiment, we tested the antibody in

renal tissue from *sgk1*$^{-/-}$ mice, where the 48 kDa band was missing. However, the antibody binds to further proteins, as evidenced from additional bands in the Western blot. After incubation with a horseradish peroxidase (HRP)-conjugated anti-rabbit secondary antibody (Amersham, Freiburg, Germany) the bands were visualized with enhanced chemoluminescence (ECL) according to manufacturer's instructions. Homogenates were also probed with a primary GAPDH (Santa Cruz, Heidelberg, Germany) antibody as a loading control. Densitometric analysis of SGK1 was performed by using Quantity One software (Bio-Rad Laboratories, California, USA, 1998) and normalized using GAPDH.

III. 9 SGK1 mRNA expression: In one series of experiments, *sgk1*$^{+/+}$ wild type mice were divided into 4 groups (5 mice per group) and treated separately with 1) control diet; 2) 10% fructose for 3 weeks; 3) 4% NaCl diet for 2 weeks; 4) 10% fructose for 3 weeks plus 4% NaCl diet for 2 weeks. 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 Reverse Transcriptase M-MuLV (Roche Diagnostics GmbH, 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 GmbH, Roche Applied Science, Mannheim, Germany). 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, 2 µl cDNA Master Sybr Green I mix (Roche Molecular Biochemicals, Mannheim, Germany). The transcript levels of the housekeeping gene GAPDH 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 versus house keeping gene transcripts.

III. 10 *In situ* hybridization: For *in situ* hybridization of SGK1 mRNA**,** control and fructose treated (10% fructose for 3 weeks) 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 phosphate buffered saline (PBS, 0.1 M, pH 7.4), slides were incubated with TE buffer (100 mM Tris, 50 mM EDTA, pH 8) containing 2 µg/ml proteinase K for 10 min at room temperature and rinsed again three times with PBS. In order to reduce nonspecific background, slides were acetylated with TEA buffer (0.1 M triethanolamine, pH 8.0) containing 0.25% (v/v) acetic anhydride (Sigma) twice for 5 min. After pre-hybridization with hybridization buffer (50% formamide (Sigma), 10 % dextrane sulfate, 5 mM EDTA, 20 mM Tris pH 8, 10 mM DTT, 1x Denhardt's solution, 0.05 % tRNA, 300 mM NaCl) for 1 h at 62° C, sections were incubated with fresh hybridization buffer containing the denatured DIG-labeled sense or antisense probe (200 ng/ml) overnight at 63° C. After hybridization, slides were briefly rinsed in 2x SSC at room temperature and 3 times in 0.1x 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, 1:2500 Roche) in blocking buffer containing 0.1% Triton® X-100) for 1 h. Following 4 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, 50 mM $MgCl_2$). The color development was carried out with freshly prepared substrate solution (nitroblue tetrazolium salt (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (X-phosphate) (Roche) in Tris buffer pH 9.5). After 3 washes with PBS, slides were rinsed in distilled water, dried, and coverslipped with Kaiser's solution (Merck, Darmstadt, Germany).

III. 11 *In vivo* clearance studies: To investigate the role of SGK1 in the antinatriuretic action of insulin, male *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice were subjected to renal clearance experiments as described before (Huang et al., 2004; Vallon et al., 2005). Briefly, mice were anaesthetized using 100 mg/kg i.p. Inactin (Sigma-Aldrich Chemie, Steinheim,

Germany) and 100 mg/kg i.m. 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 into 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% of body weight. Continuous infusion was maintained at a rate of 450µl/h/30g body weight 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, ^{3}H-inulin and sodium concentrations. Blood was obtained in the middle of the basal period for measurement of blood glucose, ^{3}H-inulin and sodium concentrations. After the basal period, all mice received a loading dose of 20mU/kg body weight insulin (Novo Nordisk Pharma, Mainz, Germany) i.v. followed by a continuous infusion of 2 mU/kg/min for 60 min. This insulin regimen has previously been shown to produce antinatriuresis in rats (Gupta et al., 1992). At the onset of the insulin infusion, a 25% glucose infusion was started at the rate of 3µl/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.

III. 12 Data analysis and statistics: Data are provided as means ± SEM, n represents the number of independent experiments. All data were tested for significance using ANOVA and only results with $P < 0.05$ were considered statistically significant.

IV. RESULTS

IV. 1. Study 1: Influence of SGK1 on renal function and systolic blood pressure during high fat diet and high salt diet

IV. 1. 1 Body weight, plasma electrolytes, and intake of food, fluid and electrolytes: Body weight was not significantly affected by replacement of tap water with 1% NaCl in *sgk1*$^{-/-}$ or *sgk1*$^{+/+}$ mice under standard diet. High fat diet alone caused a similar increase of body weight in both genotypes. The additional salt load (1% NaCl) during high fat diet led to a further increase of body weight, an effect reaching statistical significance only in *sgk1*$^{+/+}$ mice (Table 2). Replacement of tap water by 1% NaCl increased fluid intake in *sgk1*$^{-/-}$ mice under both, control diet and high fat diet, but in *sgk1*$^{+/+}$ mice only under control diet. Fluid intake did not significantly increase in *sgk1*$^{+/+}$ mice under high fat diet following additional salt load. Thus, during high fat diet, fluid intake following replacement of tap water with 1% saline was significantly higher in *sgk1*$^{-/-}$ than in *sgk1*$^{+/+}$ mice. High fat diet decreased and 1% NaCl increased food intake in both genotypes. Under combined high fat and high salt diet, daily intake of Na^+ and Cl^- was significantly larger in *sgk1*$^{-/-}$ than in *sgk1*$^{+/+}$ mice. Replacement of tap water with 1% saline did not significantly affect packed cell volume or plasma Na^+ and Cl^- concentrations. However, high fat diet alone significantly increased plasma Cl^- concentrations in both genotypes (Table 2). Under combined high fat / high salt intake, plasma K^+ concentrations were significantly higher in *sgk1*$^{-/-}$ mice than in *sgk1*$^{+/+}$ despite the higher plasma aldosterone concentrations in *sgk1*$^{-/-}$ mice (Table 2).

Table 2: Body weight, intake of fluid, food, Na^+, Cl^- and K^+ **,** packed cell volume, Na^+, Cl^- and K^+ concentrations in plasma prior to and following 25 days 1% NaCl intake in mice treated with standard diet or high fat diet for 17 weeks.

	Standard Diet			
	Water		1% NaCl	
	$sgk1^{+/+}$	$sgk1^{-/-}$	$sgk1^{+/+}$	$sgk1^{-/-}$
Body Weight (g)	30.2 ± 1.4	27.7 ± 1.6	31.1 ± 0.9	32.1 ± 2.1
Fluid Intake (ml/24h)	2.7 ± 0.6	3.0 ± 0.3	5.8 ± 1.5 #	4.4 ± 0.3 #
Food Intake (g/24h)	3.0 ± 0.3	2.9 ± 0.3	4.6 ± 0.4 #	4.5 ± 0.2 #
Daily Na^+ Intake (µmol/24h)	330 ± 30	318 ± 33	1494 ± 238 #	1231 ± 56 #
Daily Cl^- Intake (µmol/24h)	306 ± 28	294 ± 30	1457 ± 239 #	1195 ± 55 #
Daily K^+ Intake (µmol/24h)	551 ± 51	531 ± 55	842 ± 64 #	822 ± 45 #
Packed Cell Volume (%)	50 ± 1	48 ± 1	49 ± 1	48 ± 1
$[Na^+]$plasma (mM)	148 ± 1	148 ± 1	150 ± 3	153 ± 3
$[K^+]$plasma (mM)	4.6 ± 0.3	4.7 ± 0.3	5.9 ± 0.2 #	5.6 ± 0.4 #
$[Cl^-]$plasma (mM)	105 ± 3	105 ± 1	115 ± 3	111 ± 2

	High Fat Diet			
	Water		1% NaCl	
	$sgk1^{+/+}$	$sgk1^{-/-}$	$sgk1^{+/+}$	$sgk1^{-/-}$
Body Weight (g)	36.5 ± 2.2 §	36.7 ± 2.9 §	43.8 ± 2.3 §#	40.5 ± 4.6 §
Fluid Intake (ml/24h)	3.3 ± 0.3	3.1 ± 0.4	2.7 ± 0.4 §	4.5 ± 0.6 *
Food Intake (g/24h)	1.7 ± 0.2 §	2.0 ± 0.2 §	2.8 ± 0.4 #§	3.4 ± 0.3 #§
Daily Na^+ Intake (µmol/24h)	190 ± 17 §	220 ± 20 §	767 ± 98 §#	1142 ± 130 #*
Daily Cl^- Intake (µmol/24h)	176 ± 16 §	204 ± 18 §	745 ± 95 §#	1115 ± 129 #*
Daily K^+ Intake (µmol/24h)	317 ± 29 §	367 ± 33 §	501 ± 66 §#	618 ± 46 §#
Packed Cell Volume (%)	48 ± 3	47 ± 2	47 ± 1	44 ± 3
$[Na^+]$plasma (mM)	149 ± 1	149 ± 1	145 ± 3	150 ± 3
$[K^+]$plasma (mM)	4.1 ± 0.2	3.9 ± 0.1 §	4.7 ± 0.2 §	5.5 ± 0.2 #*
$[Cl^-]$plasma (mM)	126 ± 6 §	120 ± 9 §	115 ± 2	114 ± 2

Arithmetic means ± SEM (N=7 mice each group); § $p<0.05$ vs respective value under standard diet, # $p<0.05$ vs. respective value prior to 1% NaCl; * $p<0.05$ vs. $sgk1^{+/+}$.

IV. 1. 2 Plasma insulin and aldosterone concentrations: Under standard diet plasma insulin concentrations (Fig. 3A) and blood glucose levels ($sgk1^{-/-}$: 108 ± 10 mg/dl; $sgk1^{+/+}$: 111 ± 3 mg/dl, n = 7 each) were similar in SGK1 knockout mice (*sgk1*$^{-/-}$) and their wild type littermates (*sgk1*$^{+/+}$). The replacement of tap water by 1 % NaCl in drinking water did not significantly alter plasma insulin levels in either genotype. Plasma aldosterone concentrations were significantly higher in *sgk1*$^{-/-}$ than in *sgk1*$^{+/+}$ mice under standard diet and high fat diet alone (Fig. 3B). High salt intake significantly decreased plasma aldosterone concentrations to similarly low values in both genotypes on control diet. Treatment of the mice for 17 weeks with high fat diet did not significantly affect plasma aldosterone concentrations but led to an approximately fourfold increase of insulin concentrations (Fig. 3A) and significantly increased blood glucose levels ($sgk1^{-/-}$: 165 ± 16 mg/dl; $sgk1^{+/+}$: 152 ± 4 mg/dl, n = 7) in both, *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ mice. Subsequent replacement of tap water with 1 % saline in animals treated with high fat diet did not affect the plasma insulin concentration (Fig. 3A) or blood glucose levels ($sgk1^{-/-}$: 150 ± 6 mg/dl; $sgk1^{+/+}$: 152 ± 8 mg/dl, n = 7) but decreased plasma aldosterone concentration. Nevertheless, the plasma aldosterone concentration remained significantly higher in *sgk1*$^{-/-}$ mice during high fat plus 1 % NaCl treatment (Fig. 3B).

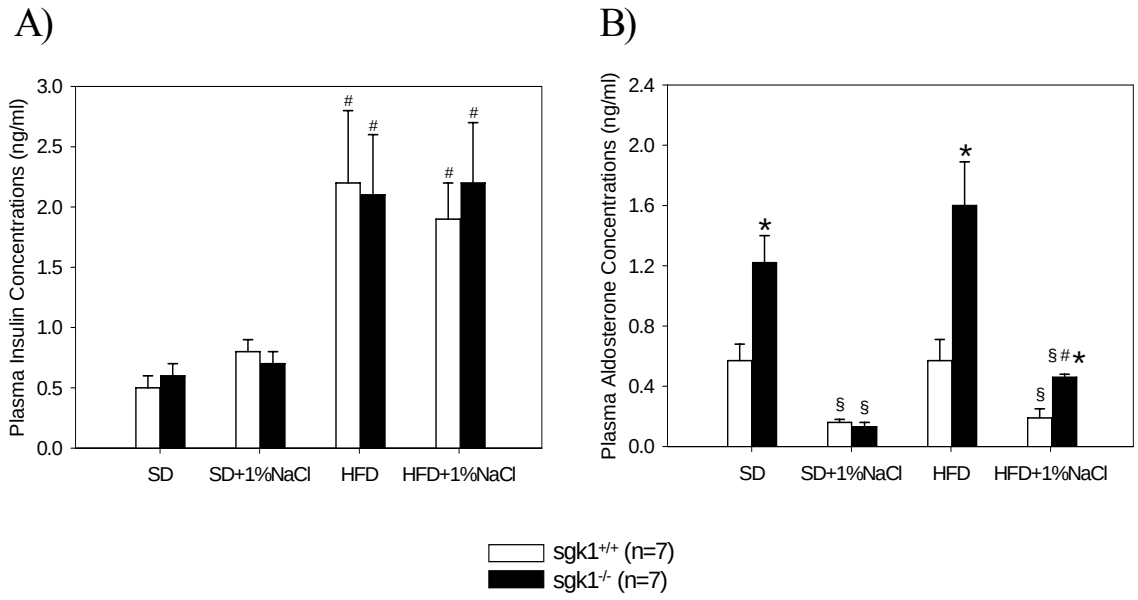

Fig. 3: Plasma insulin (A) and aldosterone (B) concentrations in sgk1$^{-/-}$ and sgk1$^{+/+}$ mice on standard diet (SD) and high fat diet (HFD) prior to and following additional 1% NaCl in drinking water.

Arithmetic means ± SEM (n = 7 each group) of plasma insulin (left) and aldosterone (right) concentrations in SGK1 knockout mice (*sgk1*$^{-/-}$, closed columns) and wild type littermates (*sgk1*$^{+/+}$, open columns). # $p<0.05$ vs. respective value under standard diet; § $p<0.05$ vs. respective value prior to 1% NaCl; * $p<0.05$ vs. *sgk1*$^{+/+}$.

IV. 1. 3 Hypoglycemic effect of insulin and glucose tolerance in mice during high fat diet: As illustrated in Fig 4, after 14 weeks on high fat diet, the decline of plasma glucose concentration following an intraperitoneal injection of insulin (0.15 U/kg body weight) was significantly decreased in mice treated with high fat diet fed for 14 weeks (Fig. 4). Moreover, the increase of plasma glucose concentration following an intraperitoneal glucose load (3 g/kg body weight) was significantly larger in animals

treated with high fat diet than in animals under control diet (Fig. 5). Accordingly, high fat diet leads to insulin resistance of cellular glucose uptake. The peak plasma glucose concentration following a glucose load was significantly higher and the decline of plasma glucose concentration following insulin administration was significantly slower in *sgk1*$^{-/-}$ than in *sgk1*$^{+/+}$ mice (Figs. 4 and 5).

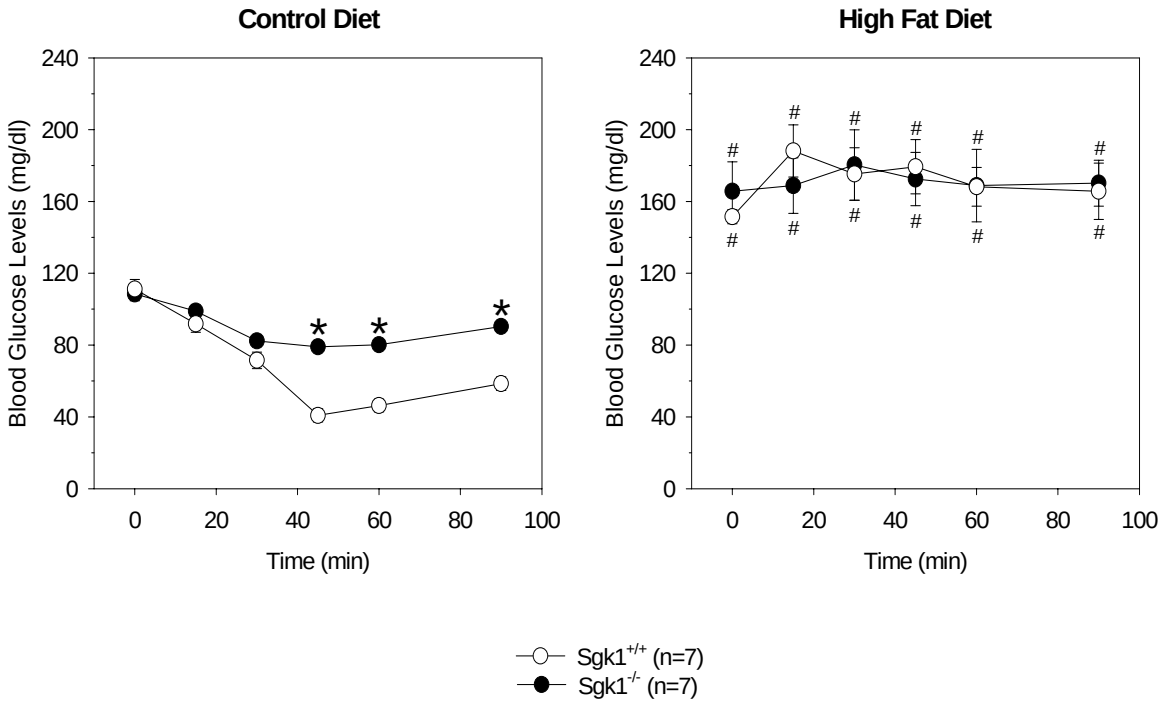

Fig. 4: Plasma glucose concentrations following intraperitoneal injection of insulin in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice.

Arithmetic means ± SEM (n = 7 each group) of plasma glucose concentrations following intraperitoneal injection of insulin (0.15 U/kg, body weight) in SGK1 knockout mice (*sgk1*$^{-/-}$, closed symbols) and wild type littermates (*sgk1*$^{+/+}$, open symbols). Experiments were performed on control diet (left panel) and on 14 weeks on high fat diet (right panel). * $p<0.05$ vs. *sgk1*$^{+/+}$, $^{\#}$ $p<0.05$ vs. respective value under standard diet.

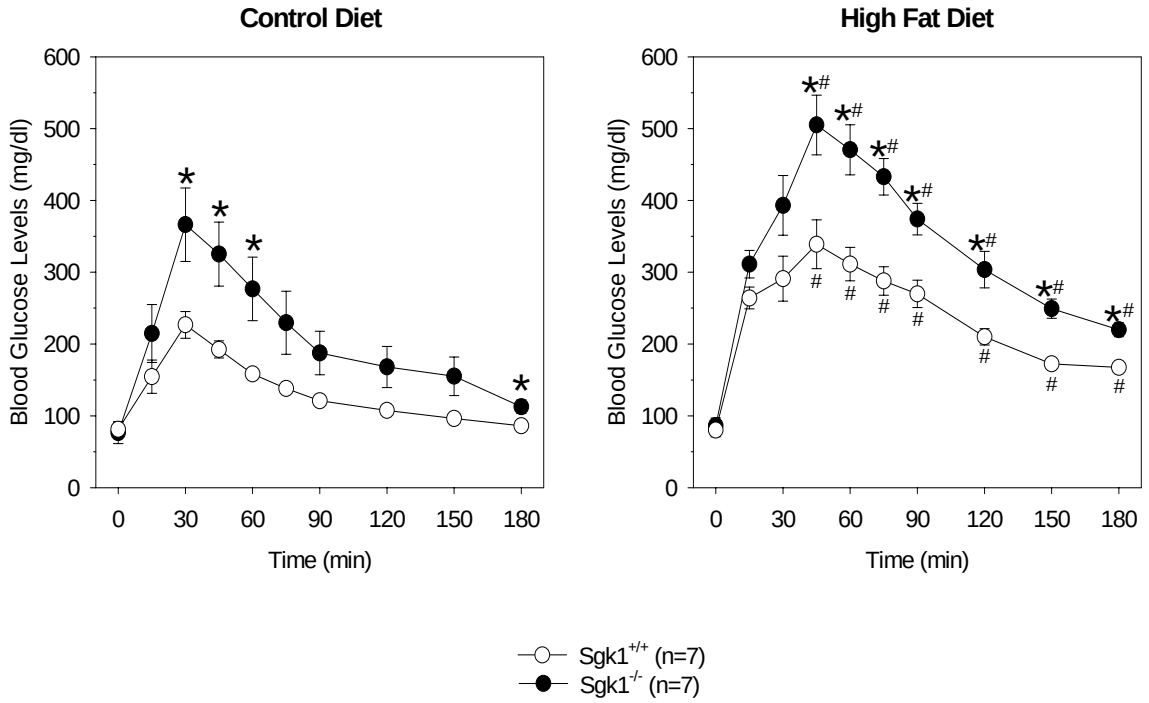

Fig. 5. Plasma glucose concentrations following intraperitoneal glucose injection in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice.

Arithmetic means ± SEM (n = 7 each group) of plasma glucose concentrations following intraperitoneal injection of glucose (3 g/kg body weight) in SGK1 knockout mice (*sgk1*$^{-/-}$, closed symbols) and wild type littermates (*sgk1*$^{+/+}$, open symbols). Experiments were performed on control diet (left panel) and on 14 weeks on high fat diet (right panel). * $p<0.05$ vs. *sgk1*$^{+/+}$, $^{\#}$ $p<0.05$ vs. respective value under standard diet.

IV. 1. 4 SGK1 protein abundance: As illustrated in Fig. 6, high fat diet significantly increased the SGK1 protein abundance in kidneys from *sgk1*$^{+/+}$ mice. Additional treatment with high salt diet led to a decrease of SGK1 protein levels which, however, tended to remain higher than under control conditions despite a marked decrease of plasma aldosterone concentration (Fig. 3B). Under standard diet replacement of tap water with 1%NaCl significantly suppressed the SGK1 protein abundance and confirmed the previous observations.

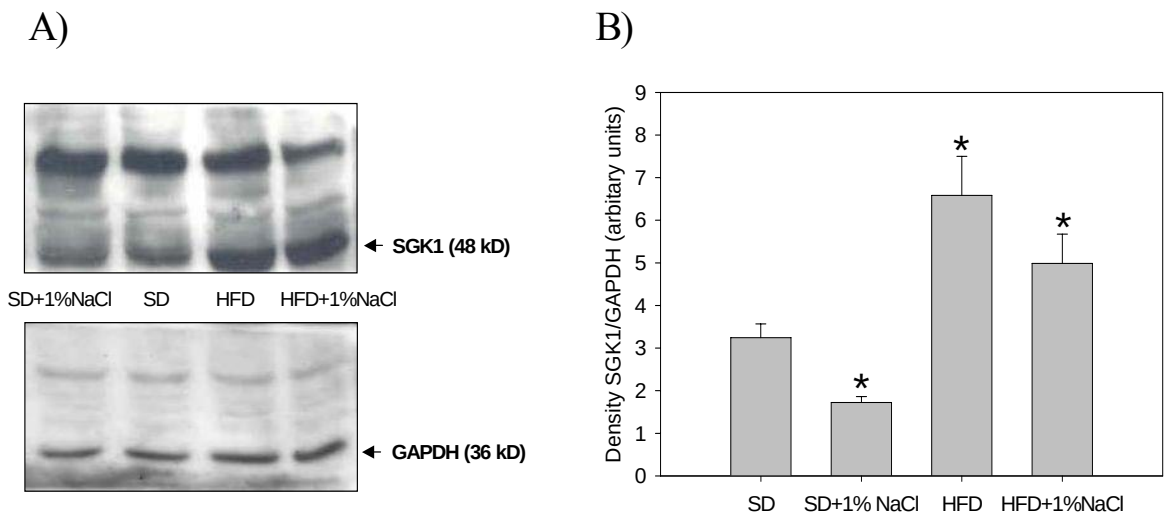

Fig. 6: Effect of high fat diet and salt excess on renal SGK1 protein expression

A. Representative Western blot of renal SGK1 protein abundance under control diet (SD), control diet + 1%NaCl (SD+1%NaCl), high fat diet (HFD) and high fat diet + 1%NaCl (HFD+1%NaCl)

B. Arithmetic means ± SEM (n = 5 each group) of renal SGK1 protein abundance in SGK1 wild type mice under control diet (SD), control diet + 1%NaCl (SD+1%NaCl), high fat diet (HFD) and high fat diet +1 %NaCl (HFD+1%NaCl). * $p<0.05$ vs. control group.

IV. 1. 5 Plasma lipid concentrations: No significant differences were observed between the genotypes in plasma total triglyceride (Fig. 7B), total cholesterol (Fig. 7A) or free fatty acid concentrations (1.4 ± 0.2 mM, n = 6, in *sgk1*$^{+/+}$ mice and 1.2 ± 0.2 mM, n = 6, in *sgk1*$^{-/-}$ mice) under control diet. High fat diet increased total plasma triglyceride concentrations about 2.5 fold in both genotypes (Fig. 7B). Additional 1% NaCl on top of the high fat diet significantly reduced total plasma triglyceride concentrations only in *sgk1*$^{-/-}$ mice. Plasma concentrations of free fatty acids were not significantly altered by either high salt diet alone (*sgk1*$^{-/-}$: 1.5 ± 0.1

mM, n = 6; *sgk1*$^{+/+}$:1.2 ± 0.2 mM, n = 6), high fat diet alone (*sgk1*$^{-/-}$: 1.5 ± 0.2 mM, n = 7, *sgk1*$^{+/+}$ 1.4 ± 0.1 mM, n = 7) or combined high fat / high salt treatment (*sgk1*$^{-/-}$: 1.6 ± 0.2, n = 6, *sgk1*$^{+/+}$ 1.5 ± 0.2, n = 6). Total serum cholesterol concentrations were significantly elevated in both *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ mice during high fat and during high fat / high salt treatments (Fig. 7A).

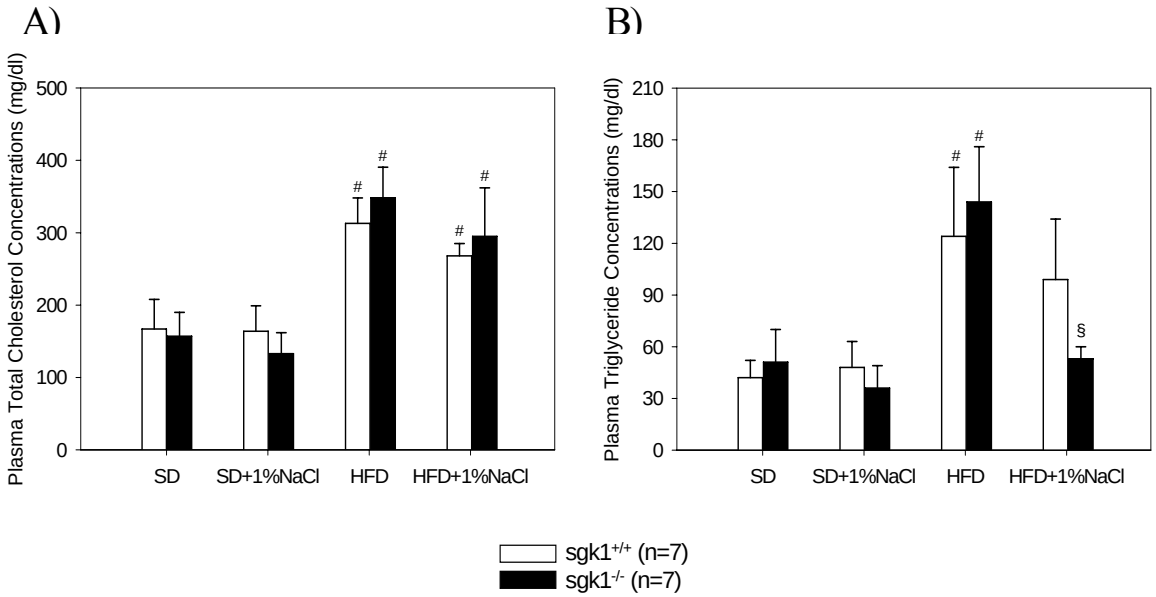

Fig. 7: Plasma total cholesterol (A) and total triglyceride (B) concentrations in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice on standard diet (SD) and on high fat diet (HFD) prior to and following additional 1% NaCl in drinking water.

Arithmetic means ± SEM (n = 7 each group) of plasma total cholesterol (left) and triglyceride (right) concentrations in SGK1 knockout mice (*sgk1*$^{-/-}$, closed columns) and wild type littermates (*sgk1*$^{+/+}$, open columns). # $p<0.05$ vs. respective value under standard diet; § $p<0.05$ vs. respective value prior to 1% NaCl.

IV. 1. 6 Systolic blood pressure: Systolic blood pressure was measured by using tail cuff method and the original blood pressure tracing was illustrated in Fig. 8. Under standard diet systolic blood pressure was similar in *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ mice (Fig. 9). Replacement of tap water with 1 % NaCl in drinking water did not significantly alter blood pressure in either genotype (Fig. 9). Exposure to high fat diet led to a marked and statistically significant increase of blood pressure which was similar in both genotypes (Fig. 9). Subsequent exposure to 1% NaCl in drinking water at continued high fat diet was followed by a significant increase of blood pressure in *sgk1*$^{+/+}$ but not in *sgk1*$^{-/-}$ mice. Accordingly, blood pressure was virtually identical in *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ animals during high fat diet and access to tap water but was significantly higher in *sgk1*$^{+/+}$ than in *sgk1*$^{-/-}$ following a 9 or 25 day additional exposure to 1% NaCl in drinking water.

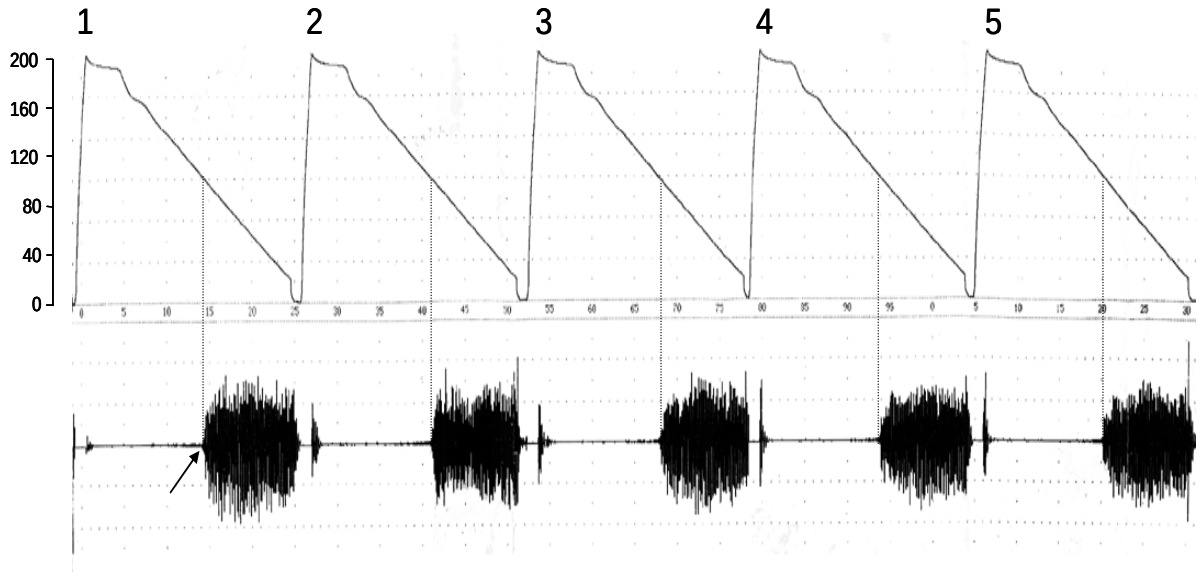

Fig. 8: Representative original blood pressure tracing measured by tail-cuff method. Precautions for tail-cuff pressure measurement were taken in the present study (see method section). Blood pressure recording was considered to be successful if the mouse did not move and a clear initial pulse (arrow) can be seen. Five tail-cuff recordings were obtained in each session. The blood pressure for the session was accepted when the deviations of the five blood pressure readings were less than 5 mm Hg.

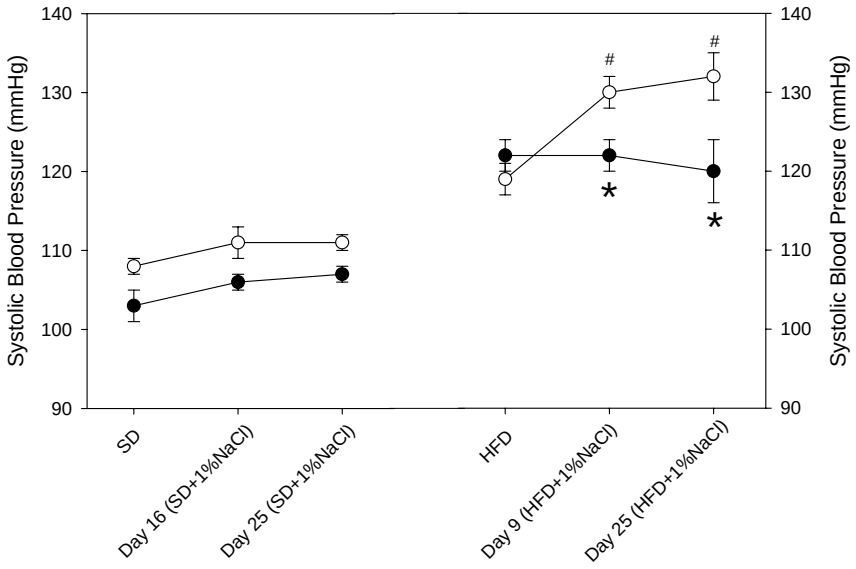

Fig. 9: Systolic blood pressure in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice on standard diet (SD) or high fat diet (HFD) prior to and following additional 1% NaCl in drinking water.

Arithmetic means ± SEM (n = 7 each group) of systolic blood pressure in SGK1 knockout mice (*sgk1*$^{-/-}$, black circles) and wild type littermates (*sgk1*$^{+/+}$, white circles) before and 9 or 25 days after addition of 1 % NaCl to drinking water. # $p<0.05$ vs. respective value prior to 1% NaCl; * $p<0.05$ vs. *sgk1*$^{+/+}$.

IV. 1. 7 Urinary excretion: The increased fluid and NaCl intake following replacement of tap water with 1% saline was paralleled by significant increases of urinary flow rate and the urinary excretions of NaCl in both genotypes and under both, standard diet and high fat diet (Fig.10). However, fluid and NaCl intake (Table 2) and urinary NaCl excretion under combined treatment with high fat diet / high salt intake were significantly larger in *sgk1*$^{-/-}$ than in *sgk1*$^{+/+}$ mice (Fig 10). Fluid intake and urinary excretion of NaCl were similar in *sgk1*$^{+/+}$ and in *sgk1*$^{-/-}$ mice under standard diet plus 1% NaCl (Fig.10).

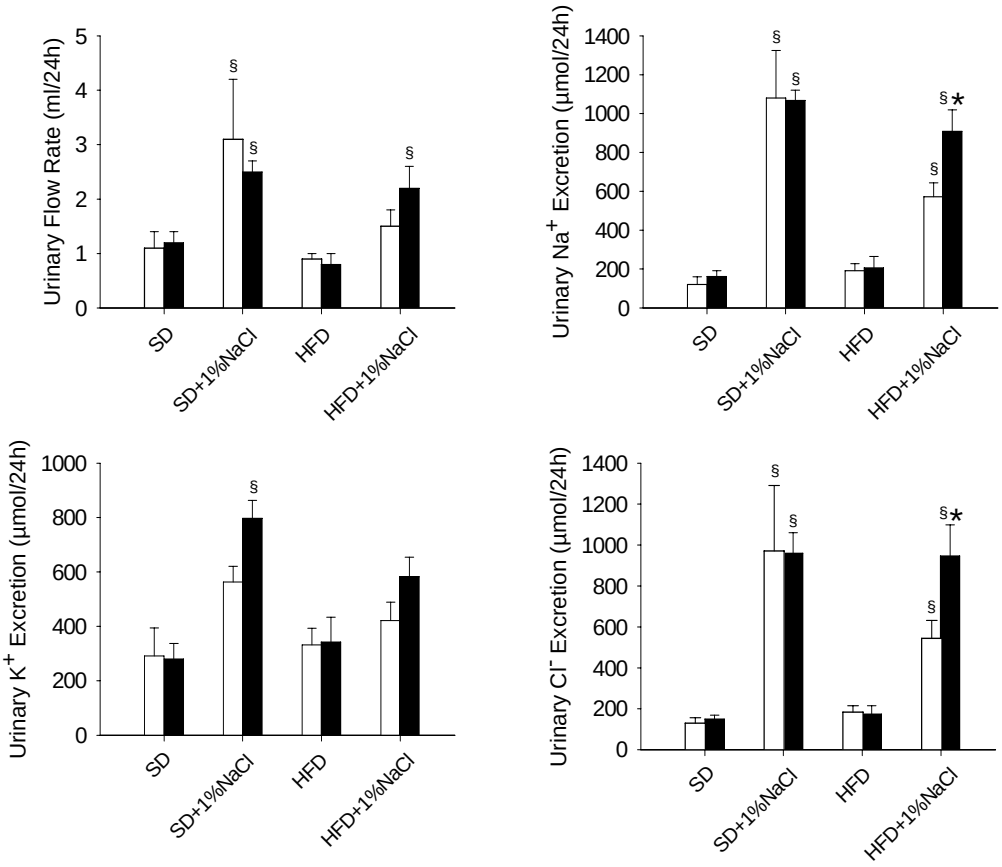

Fig. 10: Urinary excretion of water and electrolytes in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice on standard diet (SD) and high fat diet (HFD) prior to and following additional 1% NaCl in drinking water.

Arithmetic means ± SEM (n = 7 each group) of urinary flow rate, as well as urinary Na^+, K^+ and Cl^- excretions in SGK1 knockout mice (*sgk1*$^{-/-}$, closed columns) and wild type littermates (*sgk1*$^{+/+}$, open columns); § $p<0.05$ vs. respective value prior to 1% NaCl; * $p<0.05$ vs. *sgk1*$^{+/+}$.

IV. 2. Study 2: Role of SGK1 in the hypertensive effect of combined treatment with dietary fructose and salt load

IV. 2. 1 Plasma glucose, insulin and aldosterone concentrations: Under control diet blood glucose levels and plasma insulin concentrations were similar in SGK1 knockout mice (*sgk1*$^{-/-}$) and their wild type littermates (*sgk1*$^{+/+}$). As reported previously (Wulff et al., 2002), plasma aldosterone concentrations were significantly higher in *sgk1*$^{-/-}$ than in *sgk1*$^{+/+}$ mice (Fig. 11). As shown in Fig.11, high salt diet alone did not significantly alter blood glucose levels nor 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. 11). 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 significantly reduced by an additional high salt diet (Fig. 11).

IV. 2. 2 Packed cell volume, plasma Na^+, K^+ and Cl^- concentrations: Packed cell volume and plasma Na^+ concentrations were similar in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice fed on standard diet while plasma K^+ concentrations in *sgk1*$^{-/-}$ mice were significantly higher than in *sgk1*$^{+/+}$ mice (Fig. 12). Following 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. 12). 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. 12).

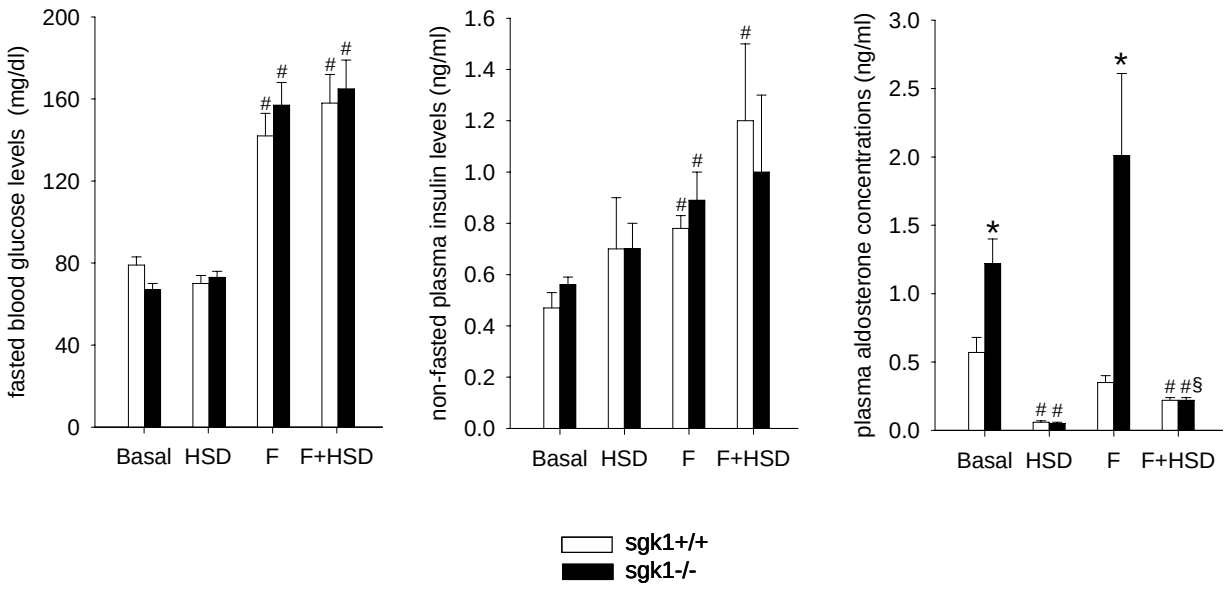

Fig. 11: P**lasma glucose, insulin and aldosterone concentrations in sgk1$^{+/+}$ and sgk1$^{-/-}$ mice on standard diet (Basal), high salt diet (HSD), fructose load (F) and fructose load + high salt diet (F+HSD).**

Arithmetic means ± SEM (N=7 each group) in SGK1 knockout mice (*sgk1*$^{-/-}$, closed columns) and wild type littermates (*sgk1*$^{+/+}$, open columns). # $p<0.05$ vs. respective value under standard diet, § $p<0.05$ vs. respective value under fructose load, * $p<0.05$ vs. *sgk1*$^{+/+}$.

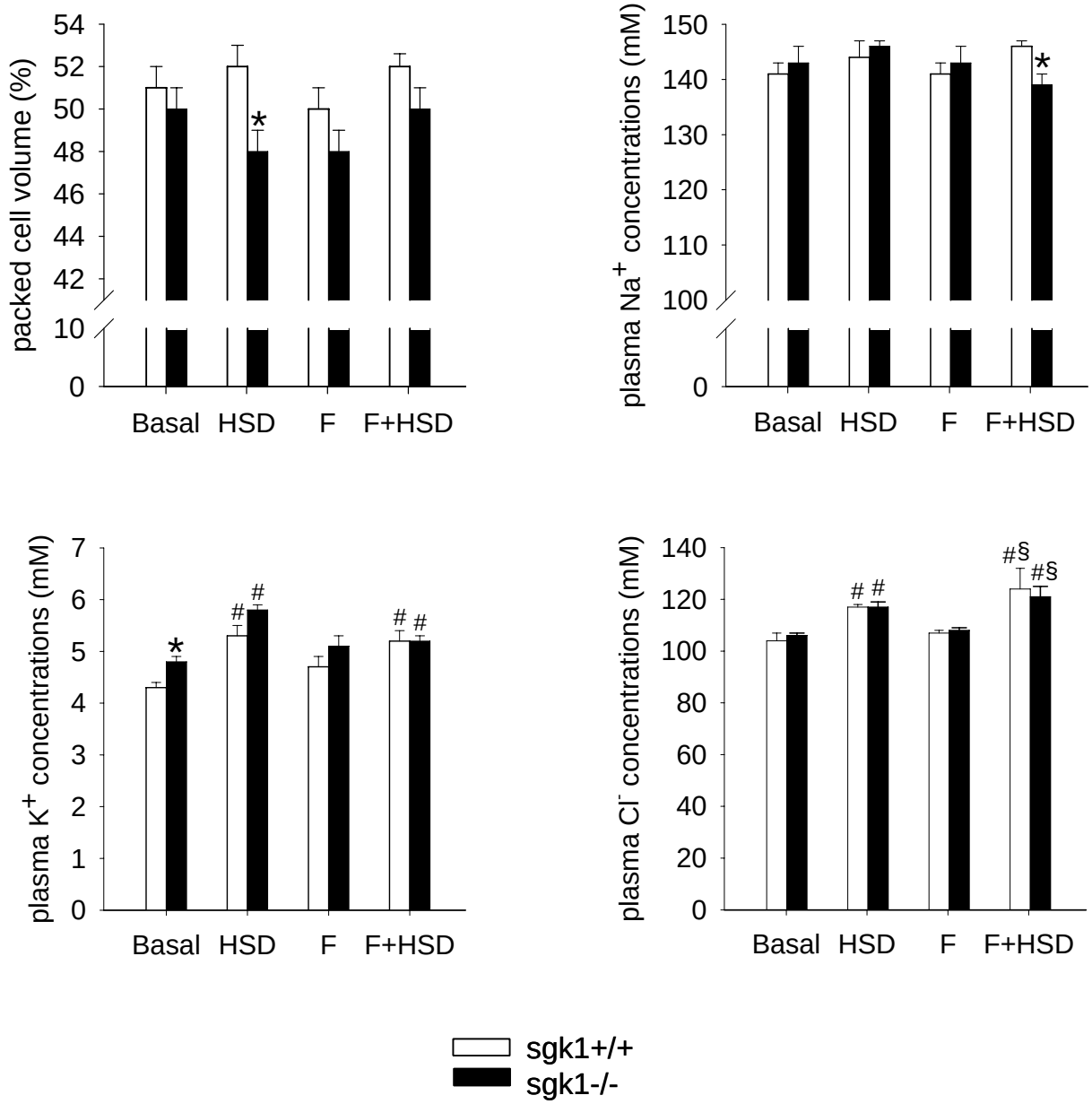

Fig. 12: Packed cell volume, plasma Na^+, K^+ and Cl^- concentrations in $sgk1^{+/+}$ and $sgk1^{-/-}$ mice on standard diet (Basal), high salt diet (HSD), fructose load (F) and fructose load + high salt diet (F+HSD).

Arithmetic means ± SEM (N=7 each group) in SGK1 knockout mice (*sgk1*$^{-/-}$, closed columns) and wild type littermates (*sgk1*$^{+/+}$, open columns). # $p<0.05$ vs. respective value under standard diet, § $p<0.05$ vs. respective value under fructose load, * $p<0.05$ vs. *sgk1*$^{+/+}$.

IV. 2. 3 Fluid intake, urinary flow rate, creatinine clearance and urinary excretion: Under control diet, fluid intake, urinary flow rate, creatinine clearance, urinary Na^+, K^+ and Cl^- excretions were similar in *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ mice (Fig. 13). In both *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice, high salt diet alone increased fluid intake, urinary flow rate, urinary Na^+, K^+ and Cl^- excretions (Fig. 13). The addition of 10 % fructose to the drinking water (for 3 weeks) led to a similar increase of fluid intake and urinary flow rate in both genotypes, and did not significantly alter creatinine clearance, urinary Na^+, K^+ and Cl^- output in neither genotype (Fig. 13). The further addition of dietary NaCl (4%) did not significantly alter fluid intake and urine volume but markedly increased urinary output of Na^+ and Cl^- in *sgk1*$^{+/+}$ mice, an effect which was enhanced in *sgk1*$^{-/-}$ mice. Thus urinary Na^+, Cl^- and K^+ excretions were higher in *sgk1*$^{-/-}$ than in *sgk1*$^{+/+}$ mice (Na^+: 2572 ± 462 vs. 1428 ± 236; Cl^-: 2364 ± 388 vs. 1379 ± 225; K^+: 679 ± 115 vs. 370 ± 60 µmol/24h, $p<0.05$ for all) following 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/24h, n=6 per group, $p<0.05$). Additional high salt diet caused a similar decrease of creatinine clearance in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice on high fructose (Fig. 13).

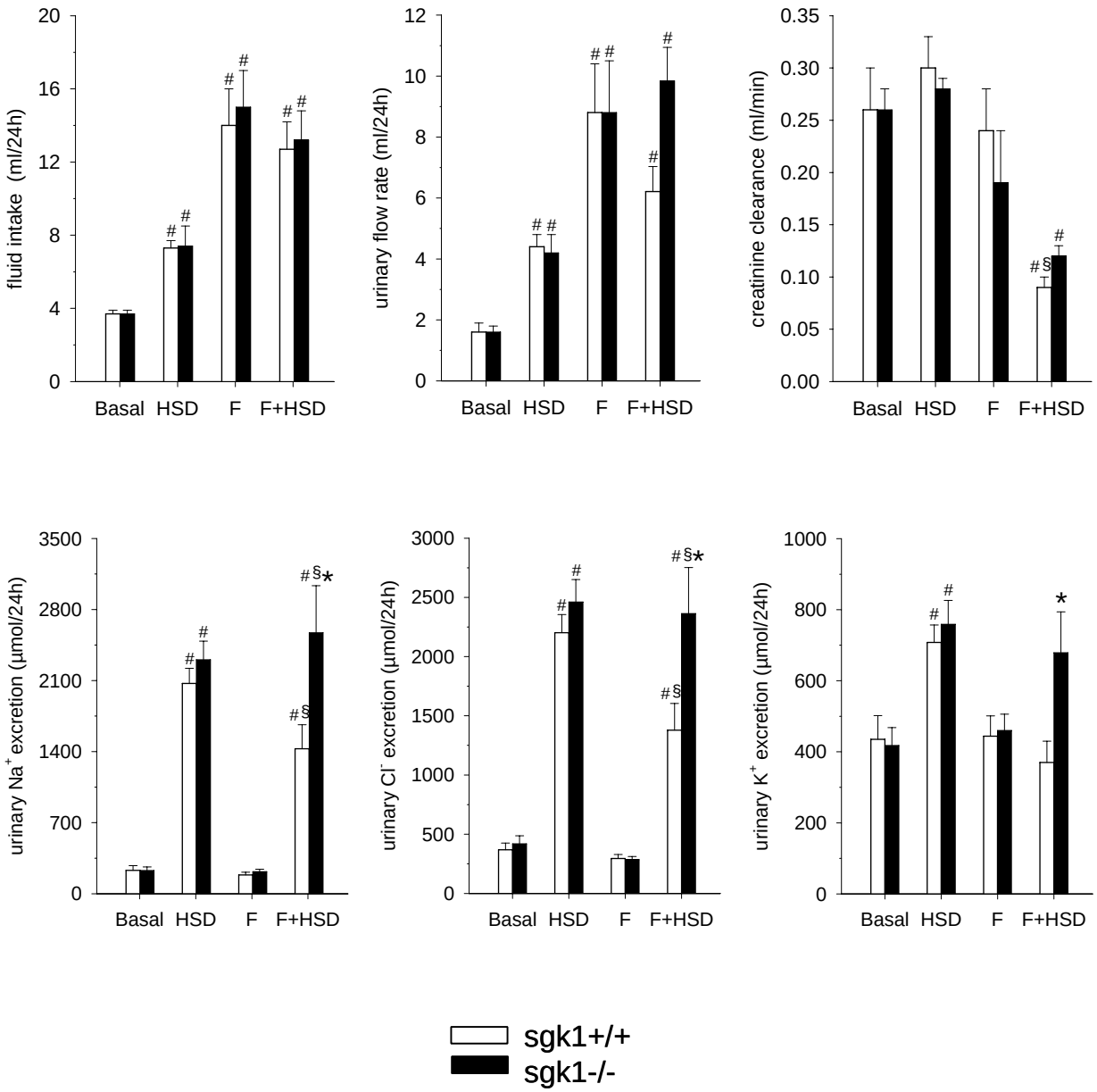

Fig. 13: Fluid intake, urinary flow rate, creatinine clerarance and urinary excretion of Na^+, K^+ and Cl^- in $sgk1^{+/+}$ and $sgk1^{-/-}$ mice on standard diet (Basal), high salt diet (HSD), fructose load (F) and fructose load + high salt diet (F+HSD).

Arithmetic means ± SEM (N=7 each group) in SGK1 knockout mice (*sgk1*$^{-/-}$, closed columns) and wild type littermates (*sgk1*$^{+/+}$, open columns). # $p<0.05$ vs. respective value under standard diet, § $p<0.05$ vs. respective value under fructose load, * $p<0.05$ vs. *sgk1*$^{+/+}$.

IV. 2. 4 Systolic blood pressure: Blood pressure was similar in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice during control diet (98.4 ± 1.9 vs. 98.2 ± 1.1 mmHg) or following administration of fructose alone for 3 weeks (99.7 ± 0.7 vs. 99.6 ± 0.7 mmHg), but significantly increased only in *sgk1*$^{+/+}$ following combined treatment with fructose and high salt intake (115 ± 1 vs. 103 ± 0.7 mmHg, $p<0.05$, Fig. 14). In contrast, without previous fructose load, high salt diet alone did not significantly affect systolic blood pressure in neither genotype (Fig. 14).

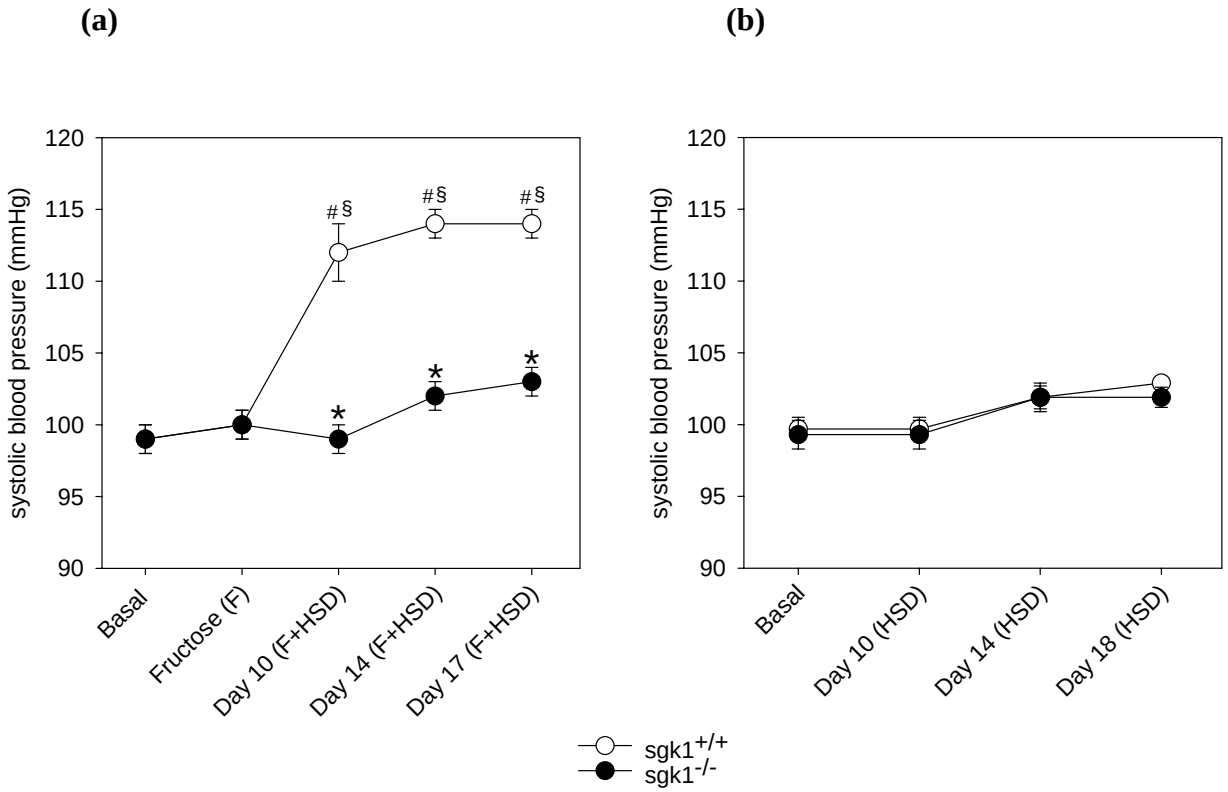

Fig. 14: Change of systolic blood pressure in sgk1$^{+/+}$ and sgk1$^{-/-}$ mice on a) standard diet (Basal), fructose load for 3 weeks (Fructose) and following 10, 14 and 17 days of fructose load + high salt diet (F+HSD); b) standard diet (Basal) and following 10, 14 and 18 days of high salt diet (HSD).

Arithmetic means ± SEM (N=6 each group) of systolic blood pressure in SGK1 knockout mice (*sgk1*$^{-/-}$, black circles) and wild type littermates (*sgk1*$^{+/+}$, white circles). # $p<0.05$ vs. respective value under standard diet, § $p<0.05$ vs. respective value under 3 weeks of fructose load , * $p<0.05$ vs. *sgk1*$^{+/+}$.

IV. 2. 5 Sgk1 mRNA expression: As illustrated in Fig. 15, 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. 11). *In situ* hybridization of SGK1 mRNA identified the glomeruli, cortical tubular structures and collecting ducts as the principal sites of SGK1 expression (Fig. 16). Fructose diet again caused a significant increase in the SGK1 expression.

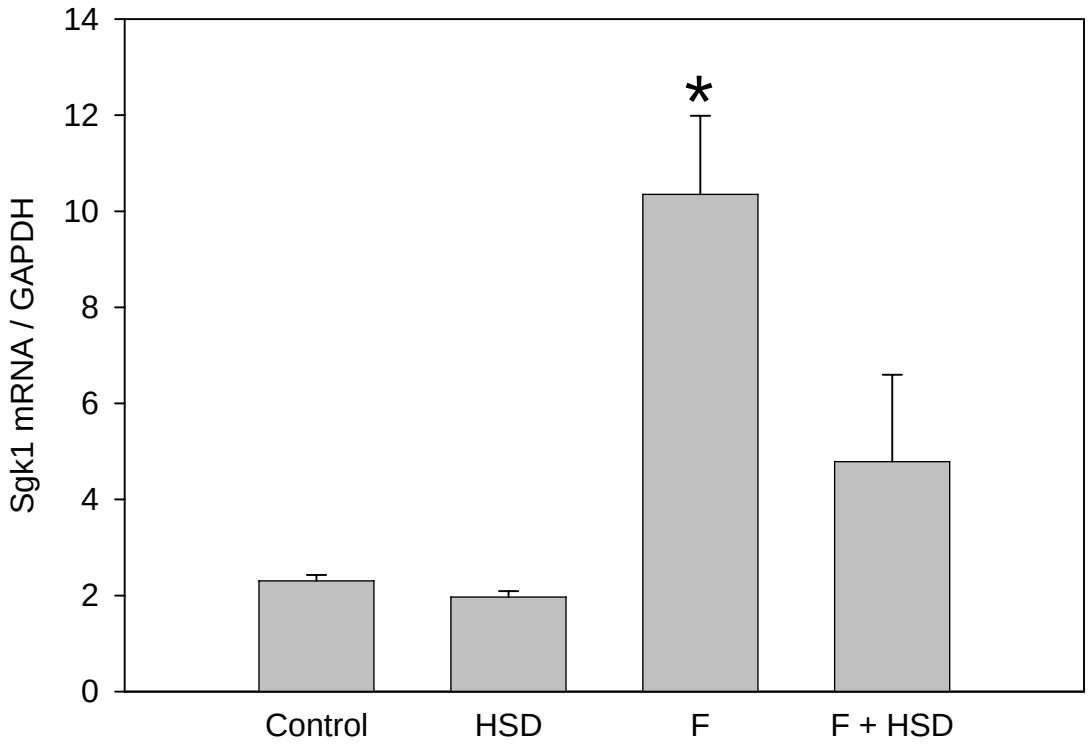

Fig. 15: Effects of control diet (Control), fructose load (F), 4% NaCl diet (HSD) and combined fructose + high salt diet (F+HSD) on renal sgk1 mRNA expression in SGK1 wild type mice.

Arithmetic means ± SEM (N=5 each group). * $p<0.05$ vs. control group

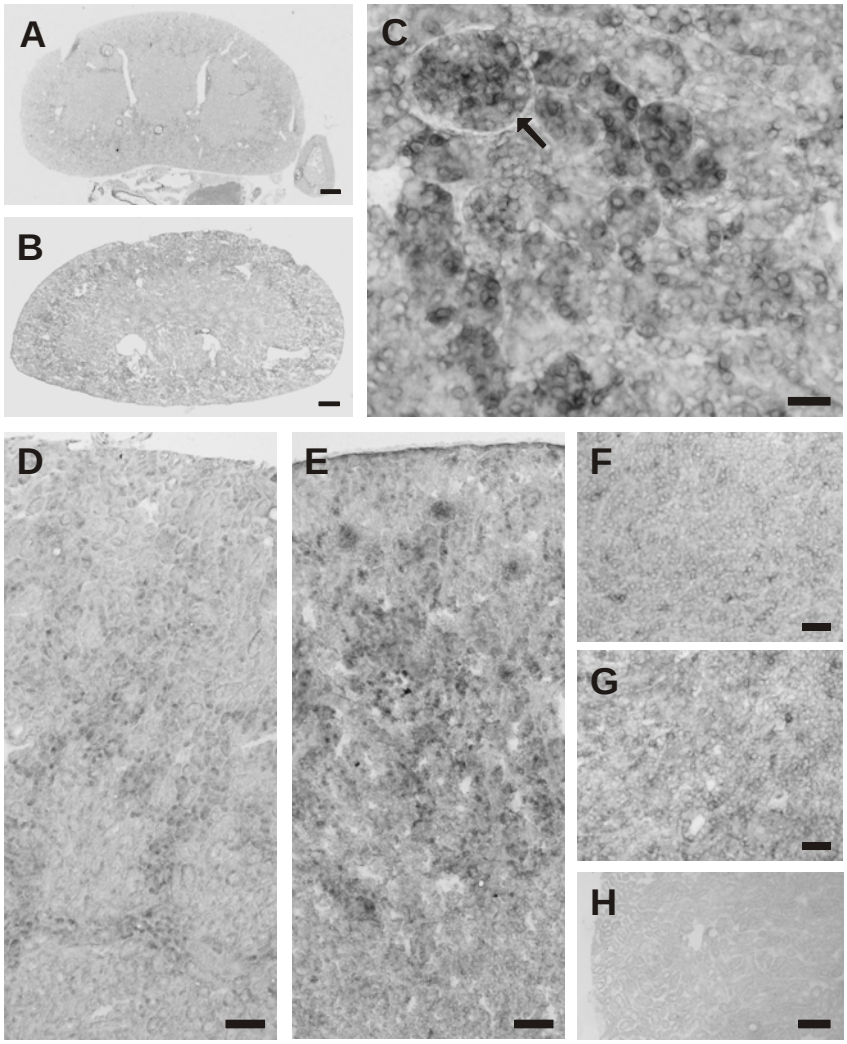

Figure 16: *In situ* hybridization of SGK1 mRNA on kidney cryostat sections of SGK1 wild type mice with and without fructose load.

A, B: Overview of SGK1 mRNA expression in the kidneys of untreated (A) and fructose-treated (B) mice. D, 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 is significantly enhanced. C: Higher magnification of the outer cortex of fructose-treated mouse. Glomerula (arrow), tubular structures and collecting ducts were strongly stained. F, 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, B = 600 µm; C = 25 µm; D, E = 100 µm; F ,G = 50 µm; H = 100 µm.

IV. 2. 6 *In vivo* insulin clearance: To further investigate the role of SGK1 in the antinatriuretic action of insulin, renal effects of intravenous insulin were determined in clearance experiments under anaesthesia. As shown in Table 3, baseline blood pressure, glomerular filtration rate (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 3). 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 nor GFR in neither genotype (Table 3).

Table 3: Mean arterial pressure, glomerular filtraion rate, fractional urinary sodium excretion and blood glucose levels in the sgk1$^{+/+}$ and sgk1$^{-/-}$ mice before and during insulin clamp (n=6).

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

Arithmetic means ± SEM; MAP: mean arterial pressure; GFR: glomerular filtration rate; Fr-$U_{Na}V$: fractional urinary sodium excretion; # $p<0.05$ vs respective values under basal, * $p<0.05$ vs sgk1$^{+/+}$.

II. 3. Study 3: Role of SGK1 in the regulation of electrolyte metabolism and blood pressure by the glucocorticoid dexamethasone

IV. 3. 1 Plasma glucose, aldosterone and chloride concentrations: As illustrated in Fig. 17, plasma glucose concentration was similar in SGK1 knockout mice (*sgk1*$^{-/-}$) and their wild type littermates (*sgk1*$^{+/+}$). Dexamethasone led to a significant and similar increase of fasting plasma glucose concentration in both genotypes. In neither genotype did additional treatment with saline significantly affect fasting plasma glucose concentration.

Food intake was not significantly different between genotypes prior to (*sgk1*$^{+/+}$: 4.2 ± 0.2 g/24 h, n = 9; *sgk1*$^{-/-}$: 4.2 ± 0.2 g/24 h, n = 9) and following treatment with dexamethasone (*sgk1*$^{+/+}$: 4.1 ± 0.3 g/24 h, n = 12; *sgk1*$^{-/-}$: 3.8 ± 0.3 g/24 h, n = 11) and with additional treatment with saline (*sgk1*$^{+/+}$: 4.1 ± 0.3 g/24 h, n = 12; *sgk1*$^{-/-}$: 3.9 ± 0.3 g/24 h, n = 12). Prior to the treatment, plasma aldosterone concentration was significantly higher in *sgk1*$^{-/-}$ than in *sgk1*$^{+/+}$ mice. Dexamethasone treatment with or without additional saline load significantly decreased the plasma aldosterone concentrations in both genotypes and dissipated the difference between the *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ mice (Fig. 17). Dexamethasone tended to decrease packed cell volume in both genotypes, an effect reaching statistical significance in *sgk1*$^{-/-}$ mice (Fig. 17). No significant differences were observed between genotypes in packed cell volume prior to and following treatment. Plasma Cl^- concentration was similar in both genotypes and was not significantly modified by dexamethasone and/or saline (Fig. 17).

IV. 3. 2 Plasma Na^+, K^+, Ca^{2+} and PO_4^{3-} concentrations: The plasma concentrations of Na^+, K^+, Ca^{2+} and PO_4^{3-} were not significantly different between *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice prior to dexamethasone treatment (Fig. 18). Dexamethasone treatment significantly decreased the plasma concentrations of Ca^{2+} in both genotypes, but the effect on plasma Ca^{2+} concentration was significantly stronger in *sgk1*$^{-/-}$ than in *sgk1*$^{+/+}$ mice. Dexamethasone treatment significantly decreased plasma PO_4^{3-} concentration only in *sgk1*$^{+/+}$ mice. Under dexamethasone treatment, the plasma Ca^{2+} concentration was significantly lower and the plasma PO_4^{3-} concentration significantly higher in *sgk1*$^{-/-}$ than in *sgk1*$^{+/+}$ mice (Fig. 18). The additional saline treatment dissipated the differences between the genotypes during dexamethasone treatment in plasma Ca^{2+} and PO_4^{3-} concentration (Fig. 18).

Saline treatment was further followed by a significant increase of plasma potassium concentration in *sgk1*$^{-/-}$ but not in *sgk1*$^{+/+}$ mice. As a result, following the combined dexamethasone/saline treatment, the plasma potassium concentration was significantly higher in *sgk1*$^{-/-}$ than in *sgk1*$^{+/+}$ mice (Fig. 18).

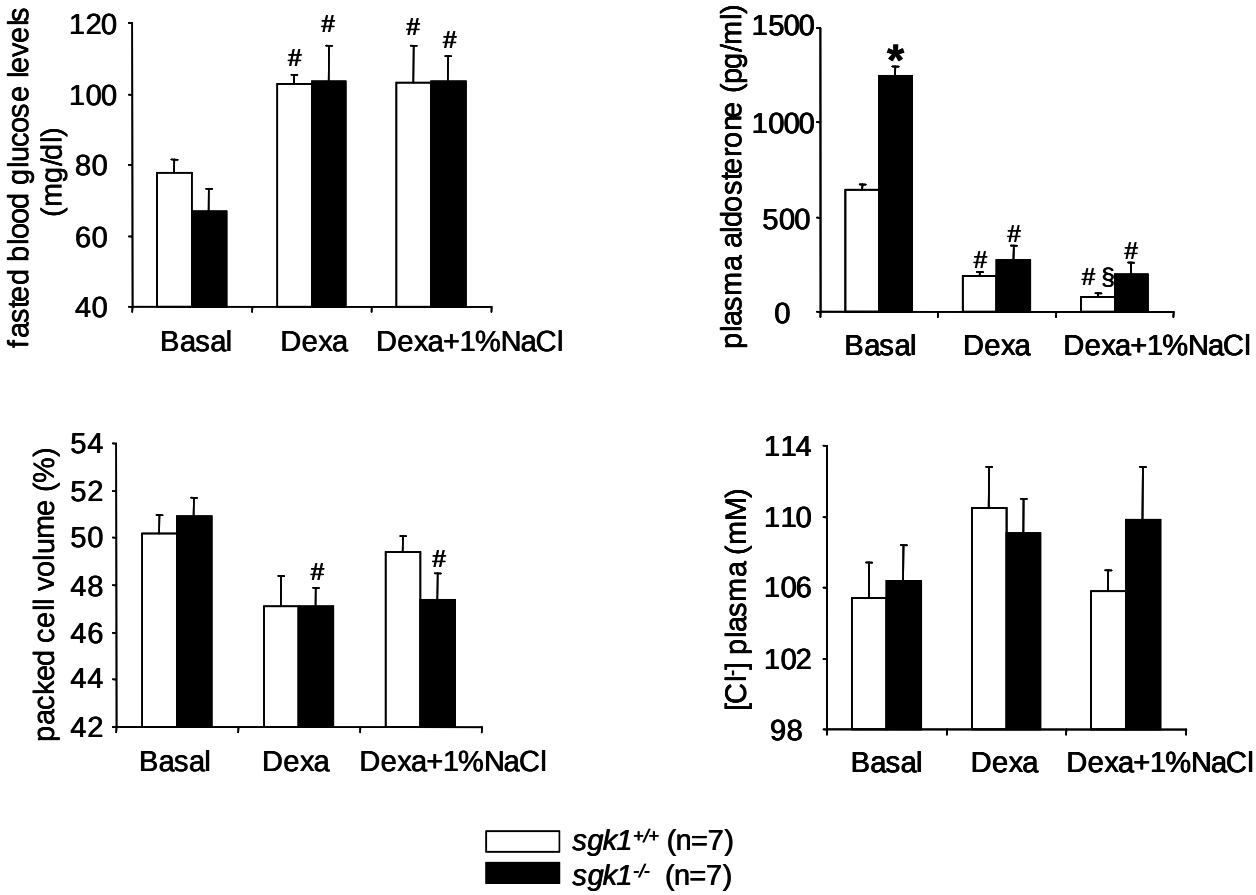

Fig. 17: Fasting plasma glucose and aldosterone concentrations, packed cell volume and plasma chloride concentration in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice prior to and following dexamethasone treatment without or with additional saline load.

Arithmetic means ± SEM (N=7 each group) of fasting plasma glucose concentration, plasma aldosterone concentration, packed cell volume and plasma chloride concentration in SGK1 knockout mice (*sgk1*$^{-/-}$, closed columns) and wild type littermates (*sgk1*$^{+/+}$, open columns) prior to and following dexamethasone treatment without or with additional saline load. $^{\#}$ $p<0.05$ vs. respective value prior to dexamethasone treatment, § $p<0.05$ vs. respective value prior to saline treatment, * $p<0.05$ vs. *sgk1*$^{+/+}$.

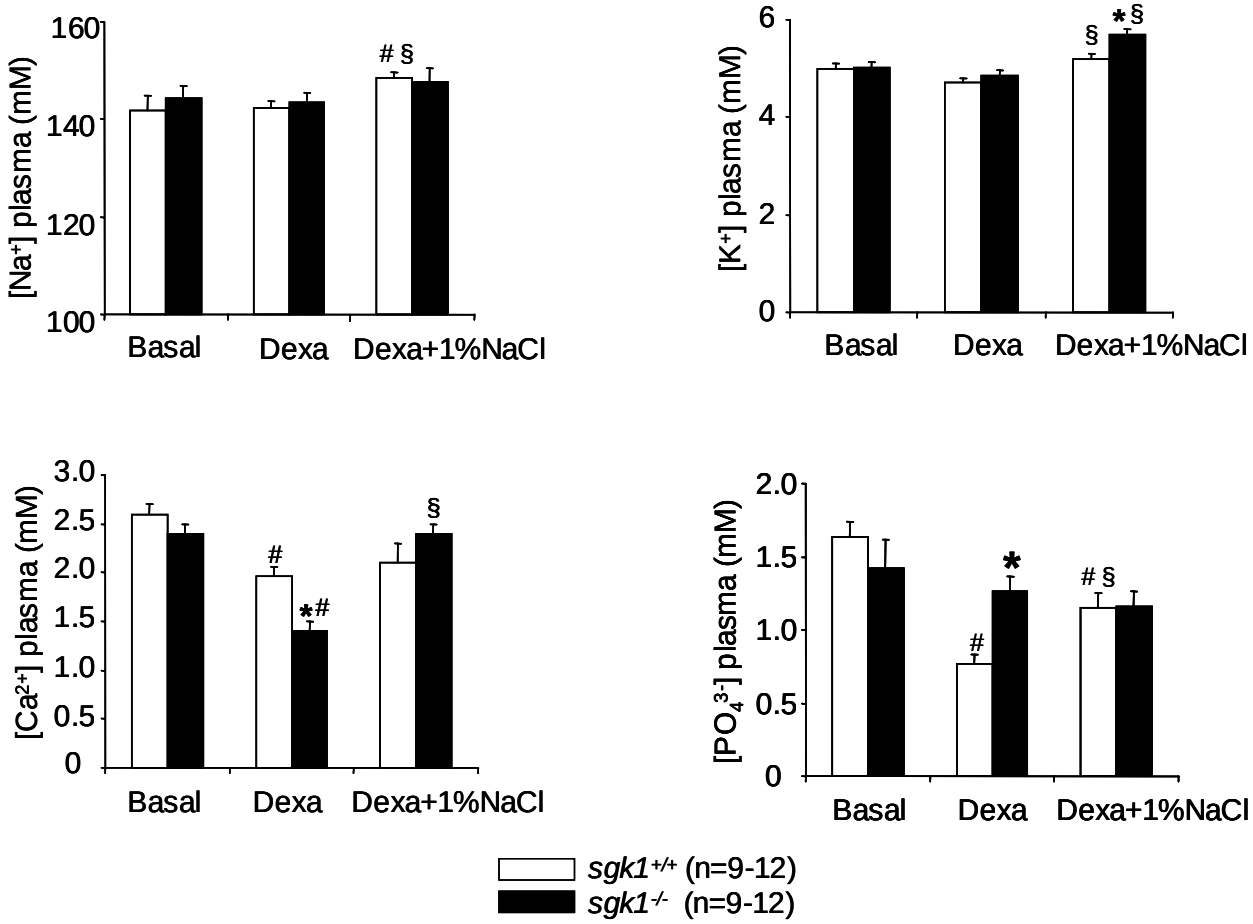

Fig. 18: Plasma concentrations of Na^+, K^+, Ca^{2+}, and PO_4^{3-} in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice prior to and following dexamethasone treatment without or with additional saline load.

Arithmetic means ± SEM (N=9-12 each group) of Na^+, K^+, Ca^{2+}, and PO_4^{3-} plasma concentrations prior to and following dexamethasone treatment (3 mg/kg, b.w., i.p.) for 14 days in SGK1 knockout mice (*sgk1*$^{-/-}$, closed columns) and wild type littermates (*sgk1*$^{+/+}$, open columns). $^{\#}$ $p<0.05$ vs. respective value prior to dexamethasone treatment, § $p<0.05$ vs. respective value prior to saline treatment, * $p<0.05$ vs. *sgk1*$^{+/+}$.

IV. 3. 3 Fluid intake, urinary flow rate, creatinine clearance and urinary protein excretion: Under control conditions fluid intake, urinary flow rate, creatinine clearance and urinary protein excretion were similar in *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ mice (Fig. 19). The administration of dexamethasone did not significantly modify fluid intake, urinary flow rate or creatinine clearance in either genotypes. Dexamethasone decreased urinary protein excretion, an effect reaching statistical significance in *sgk1*$^{+/+}$ mice (Fig.19). Additional treatment with saline significantly increased fluid intake, urinary flow rate and urinary protein excretion in *sgk1*$^{+/+}$ mice. In *sgk1*$^{-/-}$ mice only the increase of urinary flow rate reached statistical significance. At combined treatment with dexamethasone and saline, fluid intake, urinary flow rate, creatinine clearance and urinary protein excretion tended to be higher in *sgk1*$^{+/+}$ mice than in *sgk1*$^{-/-}$ mice, the differences, however, not reaching statistical significance (Fig. 19).

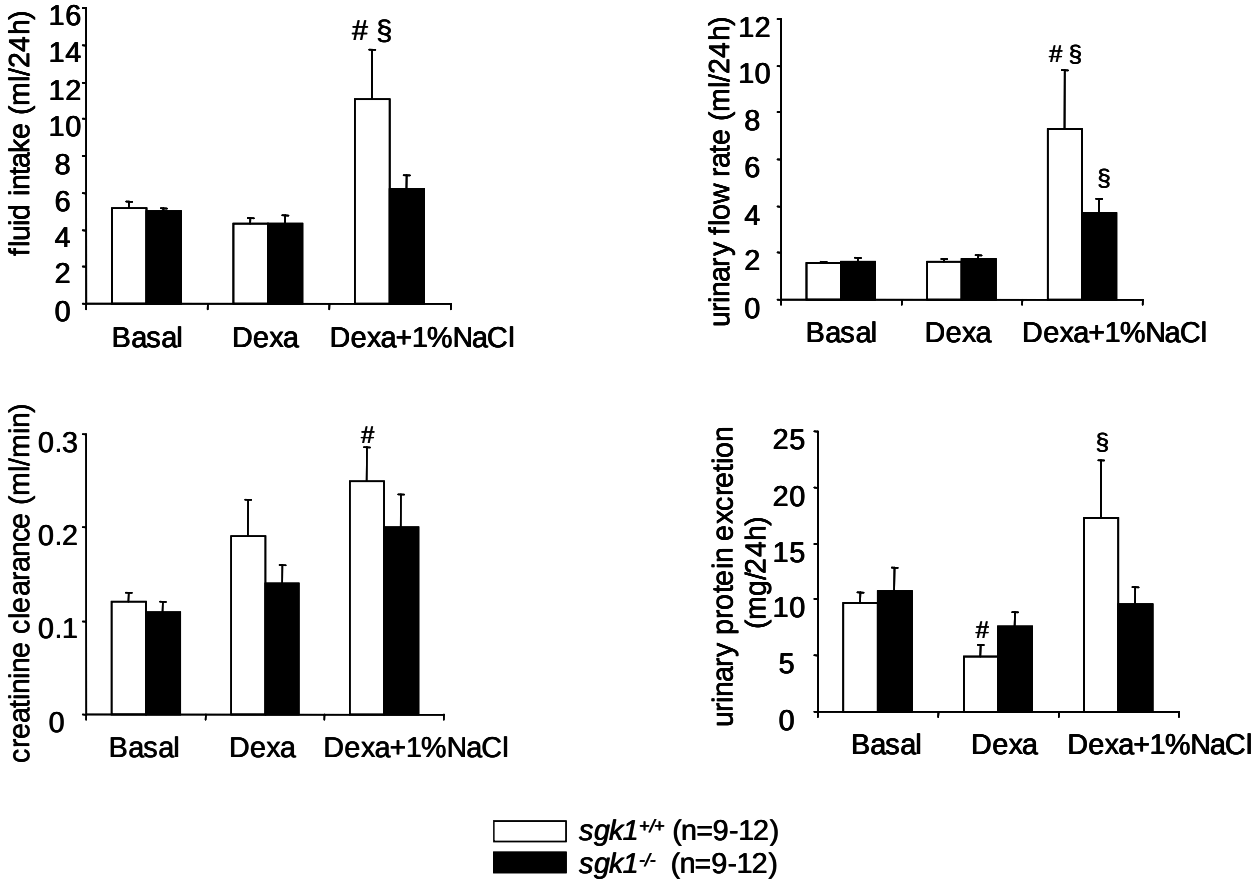

Fig. 19: Fluid intake, urinary flow rate, creatinine clearance and urinary protein excretion prior to and following dexamethasone treatment without or with additional saline load.

Arithmetic means ± SEM (N=9-12 each group) of fluid intake (ml/24 hours), urinary flow rate (ml/24 hours), creatinine clearance (ml/min) and urinary protein excretion (mg/24 hours) prior to and following dexamethasone treatment (3 mg/kg, b.w., i.p.) for 14 days in SGK1 knockout mice (*sgk1*$^{-/-}$, closed columns) and wild type littermates (*sgk1*$^{+/+}$, open columns). $^{\#}$ $p<0.05$ vs. respective value prior to dexamethasone treatment, § $p<0.05$ vs. respective value prior to saline treatment, * $p<0.05$ vs. *sgk1*$^{+/+}$.

IV. 3. 4 Urinary electrolyte excretion: Dexamethasone treatment did not significantly alter the absolute urinary excretion of Na^+ and K^+ in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice (Fig. 20). Dexamethasone treatment tended to decrease, however, the urinary Ca^{2+} excretion in *sgk1*$^{+/+}$ mice and increased urinary Ca^{2+} excretion in *sgk1*$^{-/-}$ mice. As a result, during dexamethasone treatment urinary Ca^{2+} excretion was significantly higher in *sgk1*$^{-/-}$ than in *sgk1*$^{+/+}$ mice.

Dexamethasone treatment tended to decrease urinary PO_4^{3-} excretion in both genotypes, an effect reaching statistical significance in *sgk1*$^{+/+}$ mice. The additional treatment with saline led to a significant increase of the absolute excretion of Na^+ in both genotypes but of Ca^{2+} only in *sgk1*$^{-/-}$ mice.

Similar observations were made on fractional excretions (Fig. 21). Dexamethasone treatment significantly increased the fractional urinary Ca^{2+} excretion in *sgk1*$^{-/-}$ mice to values significantly higher than those in *sgk1*$^{+/+}$ mice. Dexamethasone treatment tended to decrease the fractional excretions of K^+ and PO_4^{3-} excretion in both genotypes, an effect reaching statistical significance only in *sgk1*$^{+/+}$ mice for K^+ and in *sgk1*$^{-/-}$ mice for PO_4^{3-}. Under combined treatment with dexamethasone and saline, the fractional PO_4^{3-}excretion was significantly lower than under control conditions in both genotypes (Fig. 21).

The absolute urinary excretion of Cl^- was not significantly different between genotypes prior to (*sgk1*$^{+/+}$: 362 ± 52 µmol/24h, n=9; *sgk1*$^{-/-}$: 309 ± 39 µmol/24h, n=9) and following treatment with dexamethasone (*sgk1*$^{+/+}$: 270 ± 34 µmol/24h, n=9; *sgk1*$^{-/-}$: 294 ± 40 µmol/24h, n=9) and with additional treatment with saline (*sgk1*$^{+/+}$: 2063 ± 586 µmol/24h, n=9; *sgk1*$^{-/-}$: 1199 ± 161 µmol/24h, n=9).

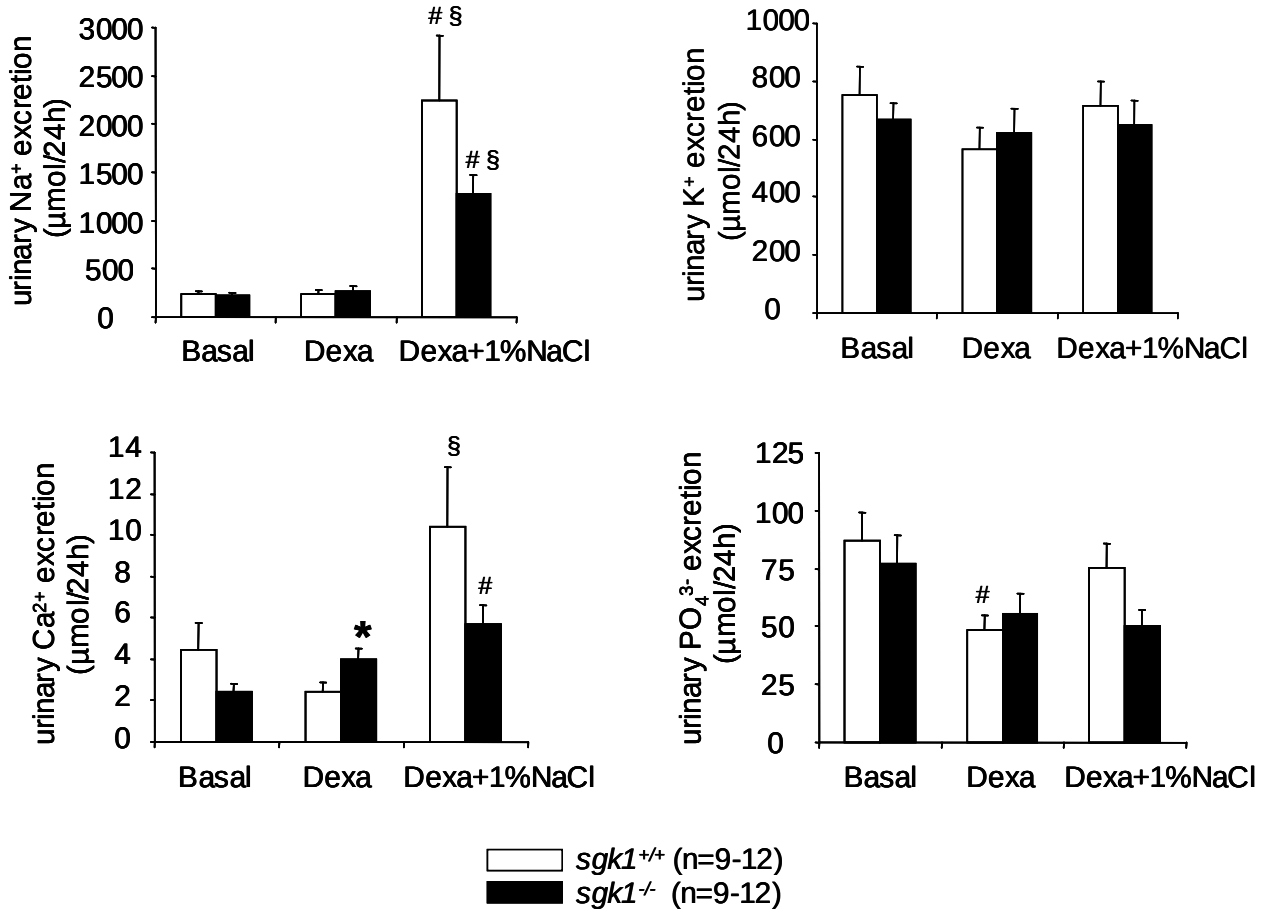

Fig. 20: Absolute urinary excretion of Na^+, K^+, Ca^{2+}, and PO_4^{3-} in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice prior to and following dexamethasone treatment without or with additional saline load.

Arithmetic means ± SEM (N=9-12 each group) of absolute (µmol/24 hours) and fractional excretions (% electrolyte clearance/creatinine clearance) of Na^+, K^+, Ca^{2+} and PO_4^{3-} prior to and following dexamethasone treatment (3 mg/kg, b.w., i.p.) for 14 days in SGK1 knockout mice (*sgk1*$^{-/-}$, closed columns) and wild type littermates (*sgk1*$^{+/+}$, open columns). $^{\#}$ $p<0.05$ vs. respective value prior to dexamethasone treatment, § $p<0.05$ vs. respective value prior to saline treatment, * $p<0.05$ vs. *sgk1*$^{+/+}$.

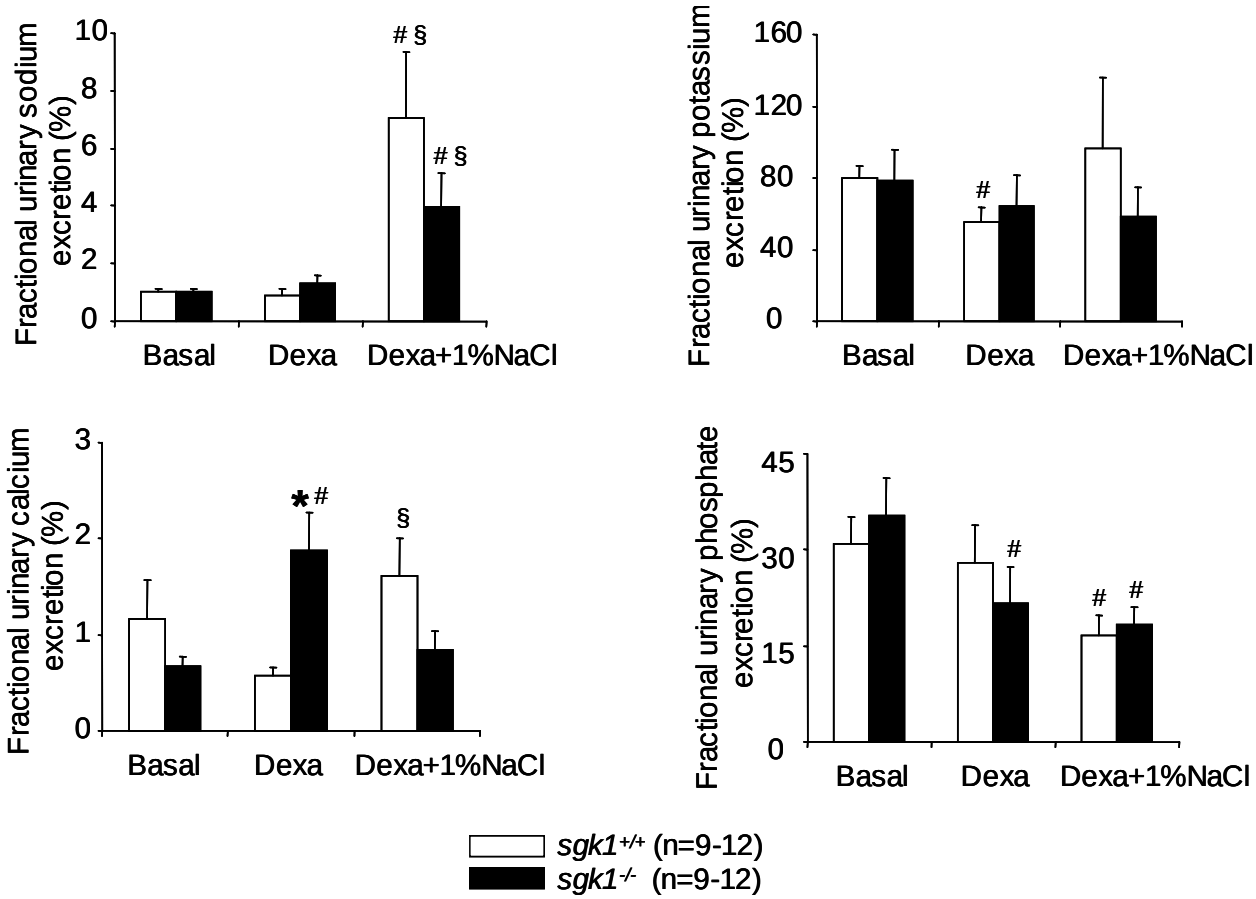

Fig. 21: Fractional urinary excretion of Na^+, K^+, Ca^{2+}, and PO_4^{3-} in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice prior to and following dexamethasone treatment without or with additional saline load.

Arithmetic means ± SEM (N=9-12 each group) of absolute (µmol/24 hours) and fractional excretions (% electrolyte clearance/creatinine clearance) of Na^+, K^+, Ca^{2+} and PO_4^{3-} prior to and following dexamethasone treatment (3 mg/kg, b.w., i.p.) for 14 days in SGK1 knockout mice (*sgk1*$^{-/-}$, closed columns) and wild type littermates (*sgk1*$^{+/+}$, open columns). $^{\#}$ $p<0.05$ vs. respective value prior to dexamethasone treatment, § $p<0.05$ vs. respective value prior to saline treatment, * $p<0.05$ vs. *sgk1*$^{+/+}$.

IV. 3. 5 Systolic blood pressure: Systolic blood pressure was similar in untreated *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice (Fig. 22). The dexamethasone treatment significantly increased the blood pressure in *sgk1*$^{+/+}$ mice and tended to increase blood pressure in *sgk1*$^{-/-}$ mice. During dexamethasone treatment, the blood pressure was significantly higher in *sgk1*$^{+/+}$ than in *sgk1*$^{-/-}$ mice. Additional saline treatment was followed by a further significant increase of blood pressure in both genotypes and dissipated the difference between *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ mice (Fig. 22).

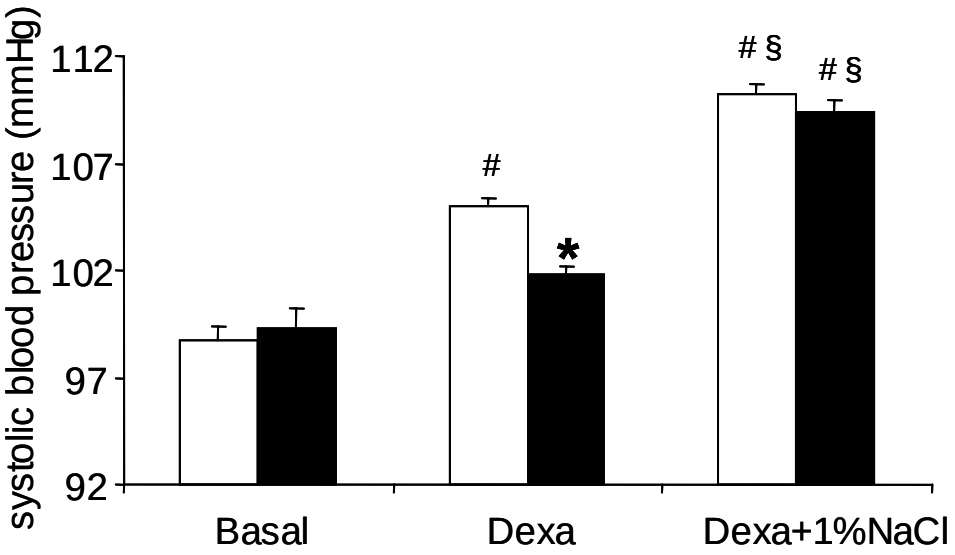

Fig. 22: Systolic blood pressure in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice prior to and following dexamethasone treatment without or with additional saline load.

Arithmetic means ± SEM (N=9-12 each group) of systolic blood pressure in SGK1 knockout mice (*sgk1*$^{-/-}$, closed columns) and wild type littermates (*sgk1*$^{+/+}$, open columns) prior to and following dexamethasone treatment without or with additional saline load. $^{\#}$ $p<0.05$ vs. respective value prior to dexamethasone treatment, § $p<0.05$ vs. respective value prior to saline treatment, * $p<0.05$ vs. *sgk1*$^{+/+}$.

IV. 4. Study 4: Role of SGK1 in salt sensitivity of glucose tolerance and peripheral glucose uptake

IV. 4. 1 Glucose tolerance tests: Glucose tolerance tests were performed in mice lacking SGK1 (*sgk1*$^{-/-}$) and in their wild type littermates (*sgk1*$^{+/+}$). Prior to 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. 23, intraperitoneal (i.p.) injection of glucose (3 g/kg b.w.) 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. 23a) and female (Fig. 23b) 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 (Figs. 23a and 23b). The differences between *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ mice was again apparent in both, male (Fig. 23a) and female (Fig. 23b) 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.

IV. 4. 2 Insulin tolerance tests: As illustrated in Fig. 24, the decline of plasma glucose concentrations following intraperitoneal injection of insulin (0.15U/kg body weight) 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.

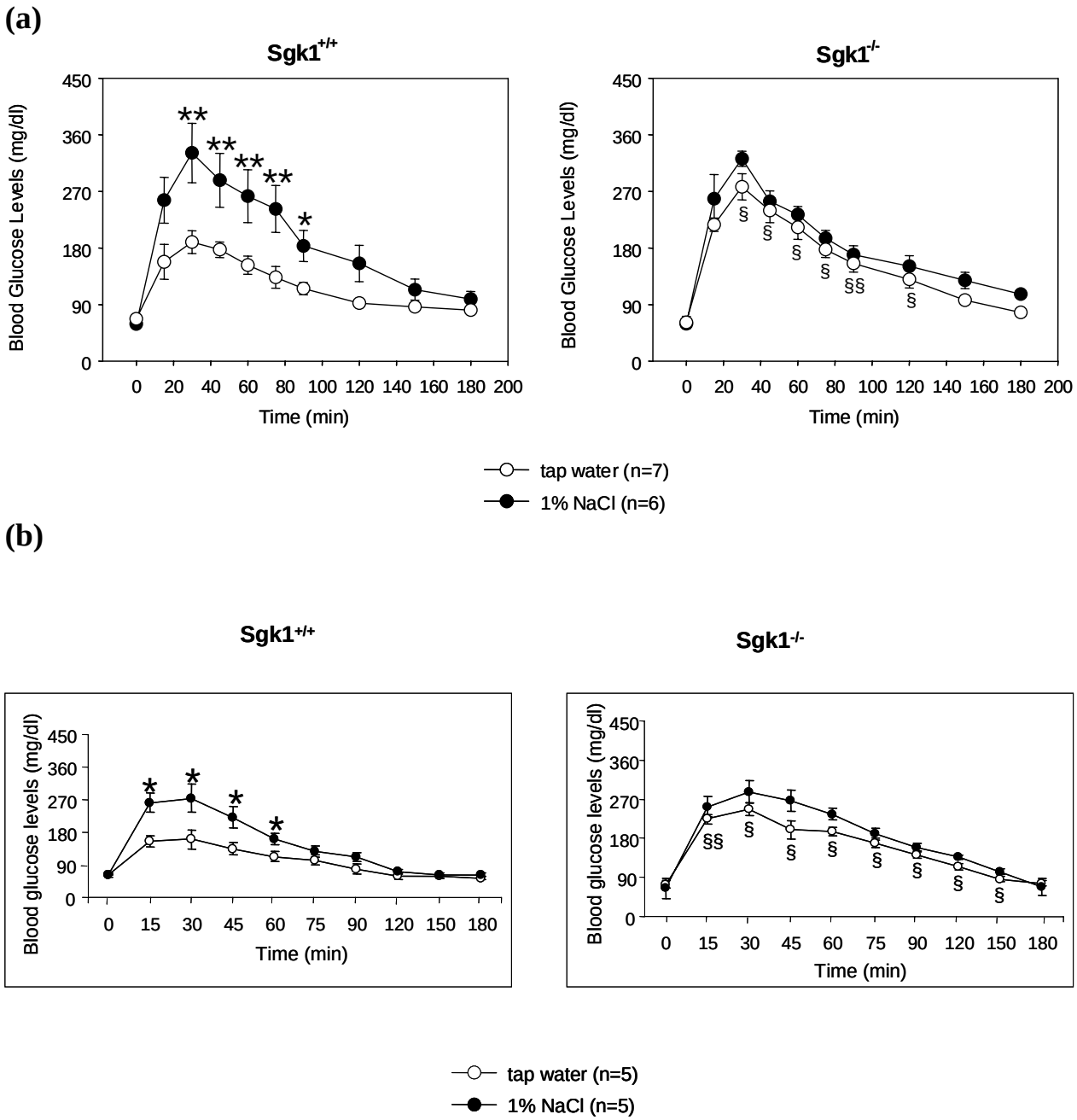

Fig. 23: Plasma glucose concentrations following intraperitoneal glucose injection in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ male (a) and female (b) mice.

Arithmetic means ± SEM (N=6 each group) of plasma glucose concentrations following intraperitoneal injection of (3g/kg body weight) glucose in SGK1 knockout mice (*sgk1*$^{-/-}$, right panel) and in wild type littermates (*sgk1*$^{+/+}$, left panel). Experiments were performed in mice drinking tap water (open symbols) and in mice drinking 1% NaCl (closed symbols) for 14 days. * $p<0.05$, ** $p<0.01$ vs. tap water drinking animals, § $p<0.05$, §§ $p<0.01$ vs. tap water drinking s*gk1*$^{+/+}$ mice.

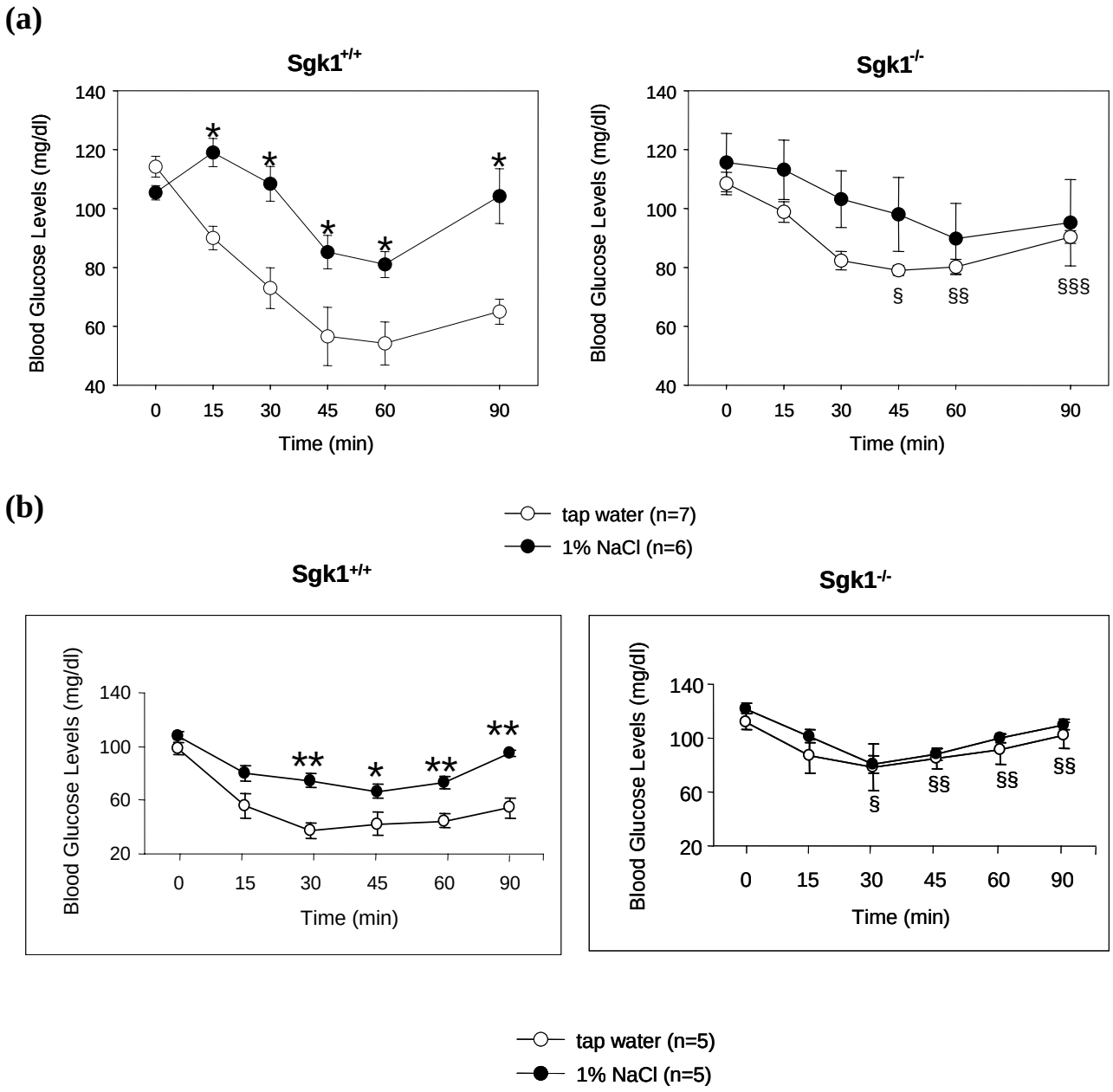

Fig. 24: Plasma glucose concentrations following intraperitoneal insulin injection in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ male (a) and female (b) mice.

Arithmetic means ± SEM (N=6 each group) of plasma glucose concentrations following intraperitoneal injection of (0.15 U/kg body weight) insulin in SGK1 knockout mice (*sgk1*$^{-/-}$, right panel) and wild type littermates (*sgk1*$^{+/+}$, left panel). Experiments were performed in mice drinking tap water (open symbols) and in mice drinking 1% NaCl (closed symbols) for 14 days. * $p<0.05$, ** $p<0.01$ vs. tap water drinking animals, § $p<0.05$, §§ $p<0.01$, §§§ $p<0.005$ vs. tap water drinking *sgk1*$^{+/+}$ mice.

IV. 4. 3 Glucose tolerance tests during mineralocorticoid excess: 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 weight) 4 hours prior to the 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. 25). 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. 25).

The subcutaneous injection of the mineralocorticoid receptor antagonist spironolactone (dissolved in soybean oil, 50 mg/kg body weight) 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 high salt diet. The increase of plasma glucose concentration was not significantly different in saline drinking *sgk1*$^{+/+}$ mice treated with DOCA + 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. 25).

IV. 4. 4 *In vivo* glucose uptake: 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 radiolabelled ^{3}2-DOG was injected into the peritoneal cavity and the radioactive tracer was determined in several tissues. As illustrated in Fig. 26, glucose uptake in muscle and fat tissue was significantly lower in saline drinking *sgk1*$^{+/+}$ mice compared to 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.

IV. 4. 5 SGK1 protein abundance: Western blot analysis was performed to determine whether the decrease of 2-deoxy-glucose uptake into skeletal muscle and fat tissue was indeed paralleled by a decrease of SGK1 protein expression following salt load. As illustrated in Fig. 27, 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 27).

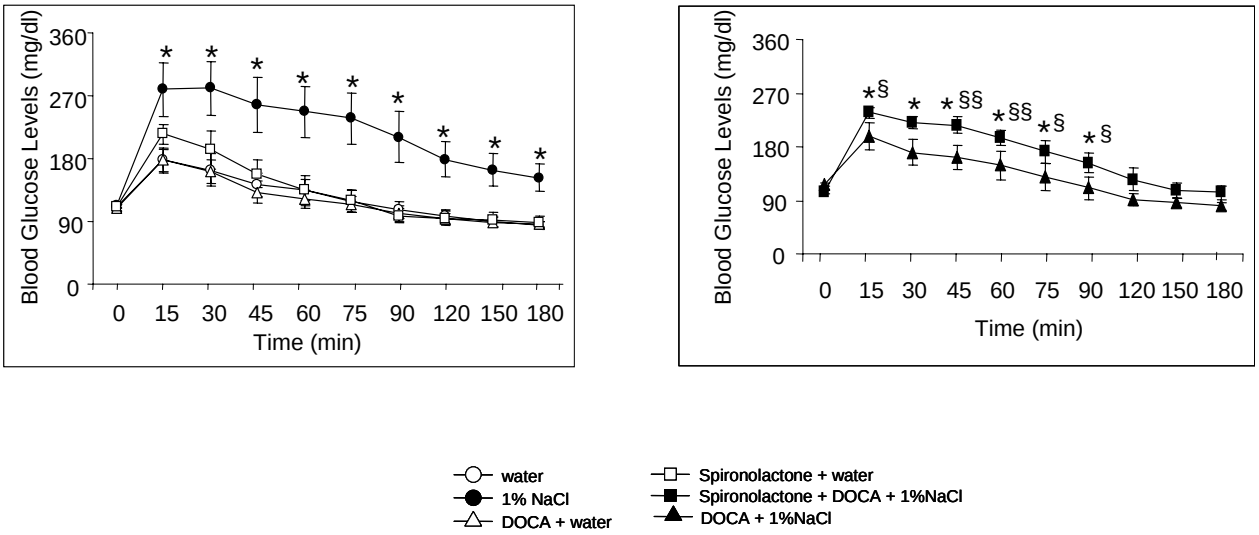

Fig. 25: Plasma glucose concentrations following intraperitoneal glucose injection in tap water drinking and 1% NaCl saline drinking wild type mice with and without prior treatment with DOCA and/or spironolactone .

Arithmetic means ± SEM (N=6 each group) of plasma glucose concentrations following intraperitoneal injection of glucose (3 g/kg body weight) 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 with circles, animals receiving DOCA prior to the glucose tolerance test are indicated by triangles, animals receiving spironolactone prior to the glucose tolerance test are indicated by squares. * $p<0.05$ vs. tap water drinking animals. § $p<0.05$, §§ $p<0.01$ vs. DOCA+1% NaCl drinking animals.

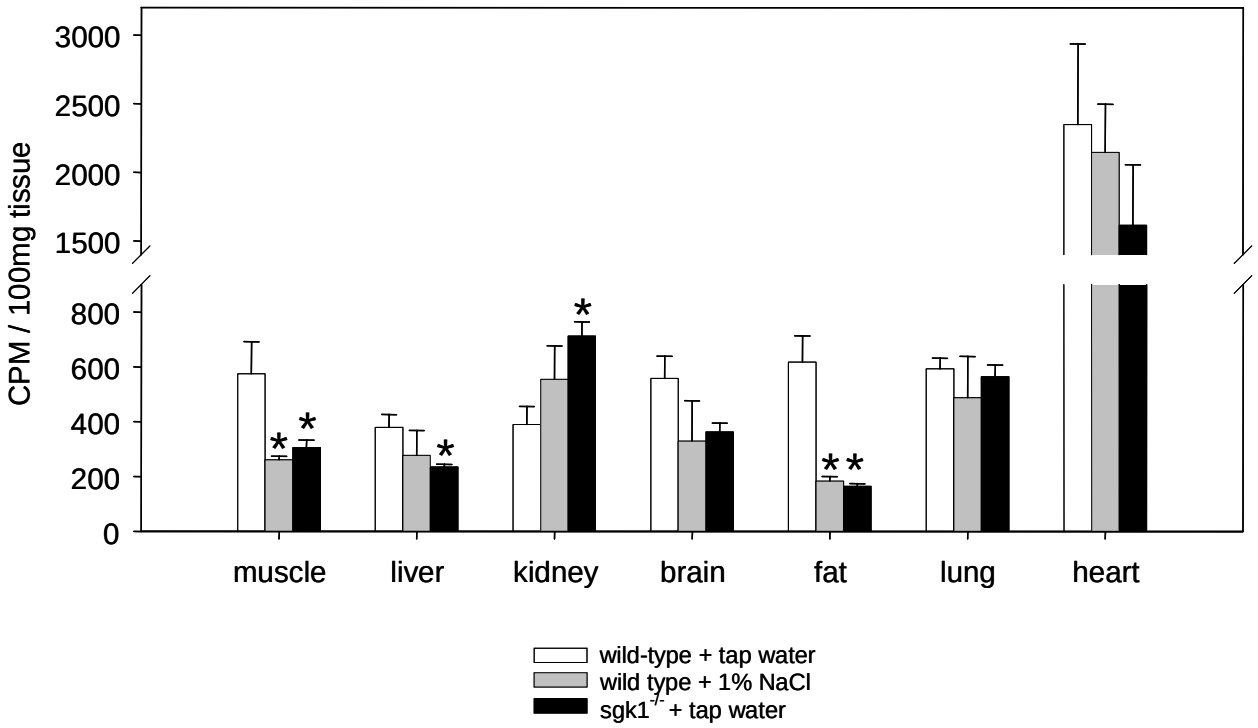

Fig. 26: ^{3}H-deoxy-glucose uptake into tissues following intraperitoneal glucose injection in tap water drinking and saline drinking *sgk1*$^{+/+}$ mice as well as in *sgk1*$^{-/-}$ mice.

Arithmetic means ± SEM (N=8 each group) of tissue radioactivity following intraperitoneal injection of radioactively labelled 2-deoxy-glucose in glucose (3 g/kg body weight). Glucose uptake has been determined in wild type mice drinking tap water (open bars), wild type mice drinking saline (grey bars) for 14 days and tap water drinking *sgk1*$^{-/-}$ (black bars). * $p<0.05$ vs. tap water drinking wild type animals.

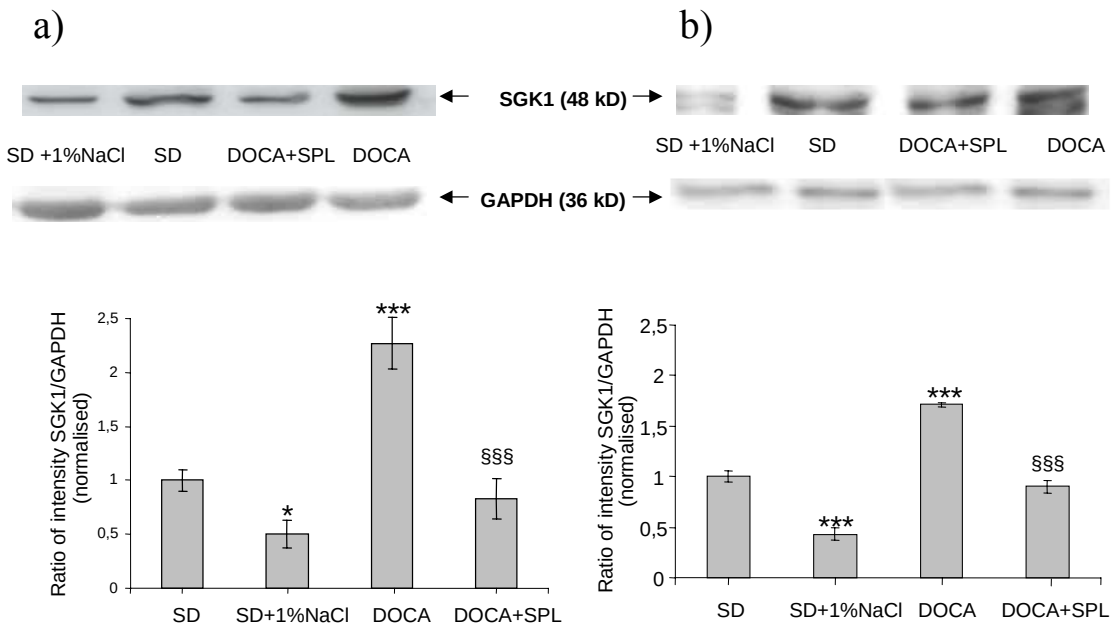

Fig. 27: Effects of control diet, 1% NaCl, DOCA and combined DOCA + spironolactone on skeletal muscle (a) and fat tissue (b) SGK1 protein abundance in *sgk1*$^{+/+}$ mice

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 ± SEM (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.

IV. 4. 6 *In vitro* glucose uptake: 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 HEK293 cells and 2-DOG uptake determined. As illustrated in Fig. 28, the transfection with active ^{S422D}SGK1 led to a marked increase of 2-DOG uptake. In contrast, transfection with inactive ^{K127N}SGK1 rather decreased 2-DOG uptake. In the presence of phloretin 2-*DOG* uptake was markedly reduced and no more sensitive to transfection with ^{S422D}SGK1 or ^{K127N}SGK1. HEK293 cells mainly express GLUT1, which is sensitive to phloretin.

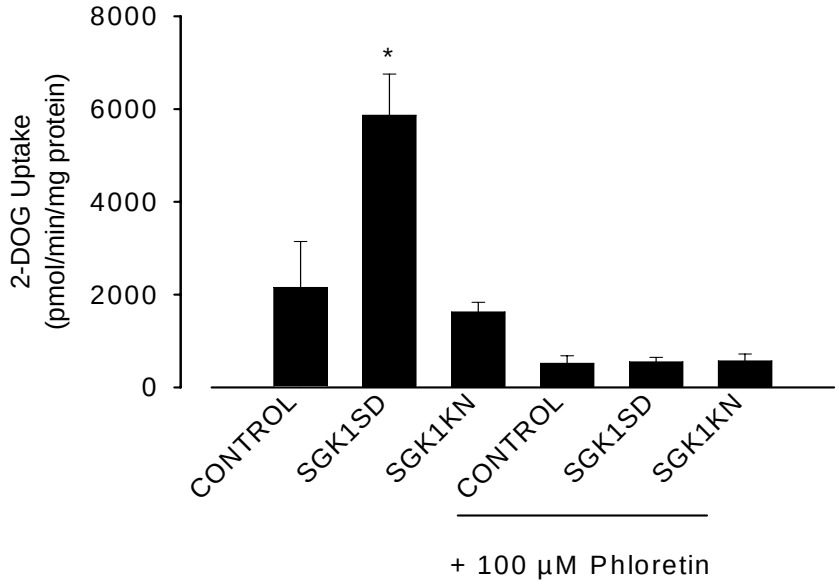

Fig. 28: Constitutively active but not inactive SGK1 stimulates ^{3}H-deoxy-glucose transport

HEK-293 cells were transfected with active ^{S422D}SGK1, inactive ^{K127N}SGK1 or empty vector. Labelled 2-DOG uptake was studied 2 days after transfection in the presence and absence of phloretin (100 µM). 2-DOG uptake was stimulated by ^{S422D}SGK1 (SGK1SD) whereas transport remained unaffected upon expression of the inactive ^{K127N}SGK1 (SGK1KN). Arithmetic means ± SEM. (N=4) * $p<0.05$ vs. transfection with empty vector (CONTROL).

V. DISCUSSION

V. 1. Study 1: Influence of SGK1 on renal function and systolic blood pressure during high fat diet and high salt diet

The present observations confirm the previous study (Wulff et al., 2002) demonstrating that under standard diet urinary salt excretion and blood pressure are similar in *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ mice. However, the elevated plasma aldosterone concentrations in *sgk1*$^{-/-}$ mice points 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 (Wulff et al., 2002).

The extracellular volume expansion during high salt intake is expected to decrease aldosterone release and thus to decrease the expression of SGK1 (Bhargava et al., 2001; Brennan and Fuller 2000; Chen et al., 1999; Djelidi et al., 2001; Loffing et al., 2001; Narey-Fejes-Toth et al., 1999; Pearce 2003; Shigaev et al., 2000; Verrey et al., 2003). Accordingly, salt excess should lower SGK1 expression in wild type mice and thus dissipate the differences of SGK1 dependent stimulation of renal tubular Na^+ transport between *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice.

Under standard diet saline drinking indeed lowers plasma aldosterone concentrations and does indeed not lead to differences in blood pressure between *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ mice. Interestingly, plasma aldosterone levels remain elevated in sgk1$^{-/-}$ mice even under high fat diet, which is paralleled by increase of blood pressure. Presumably, the increase of blood pressure during high fat diet does not translate into enhanced renal perfusion and thus remains without effect on renal renin release. Additional saline drinking decreased plasma aldosterone concentrations in both *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ mice. Again, the aldosterone plasma levels remained significantly higher in *sgk1*$^{-/-}$ mice. The difference under this experimental condition could be due to increased plasma K^+ concentrations, a well known stimulator of aldosterone release (Stanton et al., 1982). Plasma K^+ concentrations in *sgk1*$^{-/-}$ mice are at least partially enhanced due to impaired renal potassium elimination (Huang et al., 2004; Stanton et al., 1982).

In contrast to *sgk1*$^{+/+}$ mice on a standard diet, *sgk1*$^{+/+}$ mice on a high fat diet were sensitive to additional salt intake, possibly because the additional salt intake did not lead to appropriate downregulation of SGK1 transcript levels. High fat diet markedly increased the expression of SGK1 in the kidney. The present study does not address the mechanism underlying enhanced renal expression of SGK1. It is noteworthy, though, that high fat diet may lead to activation of the peroxisome proliferator-activated receptor gamma (PPARγ) (Inoue et al., 2005), which in turn has been shown to enhance SGK1 transcription (Hong et al., 2003).

Moreover, high fat diet leads to increased insulin plasma concentrations. The hyperinsulinemia is presumably a result of insulin resistance, as reflected by the decreased glucose tolerance and the blunted hypoglycaemic effect of insulin. Irrespective of the diet, the glucose tolerance and hypoglycaemic effect of insulin are significantly decreased in *sgk1*$^{-/-}$ mice, pointing to a role of SGK1 in the regulation of cellular glucose uptake. As a matter of fact, SGK1 indeed stimulates GLUT1 (Palmada et al., 2006) and glucose uptake into several tissues is blunted in *sgk1*$^{-/-}$ mice (Boini et al., 2006).

The hyperinsulinemia is expected to stimulate SGK1 and subsequently ENaC (Blazer-Yost et al., 1998; Blazer-Yost et al., 1999; Blazer-Yost et al., 2004; Faletti et al., 2002; Wang et al., 2001). Obesity is associated with peripheral insulin resistance, abnormal glucose metabolism and subsequent development of hyperinsulinemia, which in turn favours the development of salt sensitive hypertension by insulin dependent stimulation of salt and water reabsorption (Catena et al., 2003; de Paula et al., 2004; Et Atat et al., 2004; Hayashida et al., 2001; Song et al., 2004). Thus, the hyperinsulinism following high fat diet may override the effects of decreasing plasma aldosterone concentrations during high salt diet. High fat diet itself increases blood pressure in both genoptypes, an observation pointing to the participation of SGK1 independent mechanisms. However, the additional salt load further increases blood pressure in *sgk1*$^{+/+}$ but not in *sgk1*$^{-/-}$ mice, an observation disclosing the role of SGK1 in hypertension during combined excess of fat and salt. Thus, lack of SGK1 abrogates salt induced blood pressure increase only under high fat diet. The particular salt sensitivity of blood pressure during high fat diet may reflect SGK1 dependent stimulation of renal Na^+ reabsorption by insulin (Blazer-Yost et al., 1998).

Interestingly, under high fat diet, the plasma triglyceride levels tend to be lower in *sgk1*$^{-/-}$ mice than in *sgk1*$^{+/+}$ mice, a difference reaching statistical significance under

additional high salt diet. The presently available evidence does not allow any safe statements on underlying mechanisms. It is noteworthy, though, that SGK1 modifies a number of transport processes (Lang et al., 2003) and lack of SGK1 may in theory affect intestinal absorption of lipids. During high fat diet the additional administration of a salt load increased body weight only in *sgk1*$^{+/+}$ mice. Under those experimental conditions the *sgk1*$^{+/+}$ mice may retain more NaCl than the *sgk1*$^{-/-}$ mice, even though NaCl intake was larger in *sgk1*$^{-/-}$ than in *sgk1*$^{+/+}$ mice. As the electrolyte balance could not be fully determined throughout the treatment, however, no safe conclusions can be derived in regard to differences of extracellular fluid volume.

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 hyperinsulinism and salt excess. As amplified in the introduction, SGK1 participates in the regulation of ENaC and thus renal Na^+ excretion by aldosterone, insulin, and IGF-1 (Blazer-Yost et al., 1998; Blazer-Yost et al., 1999; Wang et al., 2003). While the effect of aldosterone is only partially dependent on the presence of SGK1 and effects of aldosterone and SGK1 are additive, ADH or insulin do not further stimulate ENaC in cells expressing active SGK1 (Arteaga et al., 2005). Thus, activation of ENaC by ADH or insulin fully depends on SGK1. The SGK1 dependent stimulation of ENaC is expected to increase extracellular fluid volume and thus blood pressure. DOCA treatment increases blood pressure similarly in *sgk1*$^{-/-}$ mice and in *sgk1*$^{+/+}$ mice (Vallon et al., 2006), indicating that SGK1 is not required for the hypertensive effects of acute mineralocorticoid excess. Mineralocorticoids increase salt reabsorption in part by stimulation of ENaC expression, an effect presumably not requiring SGK1. On the other hand, SGK1 transcript and protein abundance were less in Sprague-Dawley rats and greater in Dahl salt-sensitive rats on 8 % versus 0.3 % NaCl diets suggesting that SGK1 may play a role in the pathogenesis of hypertension (Farjah et al., 2003). The results of this study are in perfect agreement with our observations, that SGK1 plays a particular role in the hypertensive effects of hyperinsulinemia.

V. 2. Study 2: Role of SGK1 in the hypertensive effect of combined treatment with dietary fructose and salt load

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 volume expansion during high salt intake (Bhargava et al., 2001; Brennan and Fuller 2000; Chen et al., 1999; Djelidi et al., 2001; Loffing et al., 2001; Narey-Fejes-Toth et al., 1999; Pearce 2003; Shigaev et al., 2000; Verrey et al., 2003). 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, that under standard diet, urinary salt excretion and blood pressure are similar in *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ mice (Wulff et al., 2002).

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 favours the development of salt sensitive hypertension by increasing salt and water reabsorption in different nephron segments (Catena et al., 2003; El Atat et al., 2004; Hayashida et al., 2001; Song et al., 2004). 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 due to its stimulating effect on the renal epithelial Na^+ channel ENaC (Alvarez de la Rosa et al., 1999; Böhmer et al., 2000; Chen et al., 1999; Lang et al., 2000; Narey-Fejes-Toth et al., 1999; wagner et al., 2001) and the Na^+/K^+ATPase (Henke et al., 2002; Setiawan et al., 2002; Zecevic et al., 2004) the kinase contributes to the antinatriuretic effect of insulin (Bickel et al., 2001; Song et al., 2004; Zhang et al., 2005). Thus, SGK1 may be the primary kinase mediating the phosphatidylinositide-3 (PI3)-kinase dependent effects of insulin on renal tubular Na^+ transport (Blazer-Yost et al., 2003; Blazer-Yost et al., 2004; Tong et al.,

2004). The upregulation of SGK1 expression by mineralocorticoids (Bhargava et al., 2001; Brennan and Fuller 2000; Chen et al., 1999; Djelidi et al., 2001; Loffing et al., 2001; Narey-Fejes-Toth et al., 1999; Pearce 2003; Shigaev et al., 2000; Verrey et al., 2003) is expected to sensitize the distal nephron for the antinatriuretic effects of insulin. As expressed SGK1 requires activation through PI-3 kinase and PDK1 (Biondi et al., 2001; Kobayashi and Cohen 1999; Park et al., 1999), aldosterone may have smaller effects on renal Na^+ transport at low insulin levels.

Notably, fructose alone is not sufficient to elicit SGK1 dependent blood pressure increase. It has been shown that increase of arterial blood pressure induced by fructose feeding depends on dietary salt intake (Nishimoto et al., 2002). Our data similarly show that high fructose intake alone does not significantly alter blood pressure in mice on 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 of 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 (Blazer-Yost et al., 1998; Blazer-Yost et al., 1999; Blazer-Yost et al., 2004; Wang et al., 2001). 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 can not 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”, which has been observed before in rats (Vallon et al., 1997; Vallon et al., 1995) and patients (Miller 1997) 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 glomerular filtration rate (Vallon et al., 2002). The mechanisms involved are not understood but are independent of renal nerves (Birk et al., 2003) and angiotensin II receptor activation (Vallon et al., 1995). 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 rat (Farjah., 2003). In addition, moderately enhanced blood pressure is observed in individuals carrying a variant of the SGK1 gene, affecting as many as 5% of unselected Caucasians (Busjahn et al., 2002). In the same individuals increased body mass index and a shortening of the QT interval (Busjahn et al., 2002; Busjahn and Luft 2003) have been observed. The increased body mass index may be partially due to enhanced stimulation of the intestinal glucose transporter SGLT1 (Dieter et al., 2004), the accelerated cardiac repolarization due to enhanced activation of the cardiac K^+ channel KCNE1 (Busjahn et al., 2004; Embark et al., 2003). Thus, altered regulation of carriers and channels by SGK1 could account for the coincidence of obesity, hypertension and altered cardiac action potential (Lang et al., 2003).

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 those 5 % of the common population carrying the SGK1 gene variant. The observations suggest that SGK1 plays a critical role in the hypertensive effect of hyperinsulinism. As a gain of function, gene variant of SGK1 could simultaneously increase blood pressure and body mass index, SGK1 may indeed be one of the signalling molecules contributing to metabolic syndrome or syndrome X, a condition characterized by the coincidence of several disorders including hypertension, obesity, insulin resistance and hyperinsulinemia (Roth et al., 2003). Metabolic syndrome shares several attributes of Cushing´s syndrome, but does not require increased plasma cortisol levels (Bahr et al., 2002). Instead, the disorder may be caused by inappropriate activity of downstream signaling elements which could well include the serum and glucocorticoid inducible kinase SGK1.

V. 3. Study 3: Role of SGK1 in the regulation of electrolyte metabolism and blood pressure by the glucocorticoid dexamethasone

The present observations confirm the mild phenotype of untreated SGK1 knockout (*sgk1*$^{-/-}$) mice, i.e. the lack of major differences to their wild type littermates (*sgk1*$^{+/+}$). In previous studies significant differences in renal NaCl excretion were only observed following exposure to salt deficient diet (Wulff et al., 2002), and significant differences in renal K^+ excretion only during acute or chronic K^+ loading (Huang et al., 2004). An increase in plasma aldosterone appears sufficient to maintain normal renal NaCl reabsorption and potassium excretion under basal conditions.

In untreated *sgk1*$^{-/-}$ mice the fractional renal Ca^{2+} excretion is lower, pointing to enhanced renal Ca^{2+} reabsorption (Sandulache et al., 2006). In the present study, the absolute and fractional renal Ca^{2+} excretion was similarly lower in untreated *sgk1*$^{-/-}$ mice. The decreased renal Ca^{2+} excretion has been attributed to enhanced Ca^{2+} reabsorption in the loop of Henle, paralleling enhanced Na^+ reabsorption in this nephron segment. The enhanced Na^+ reabsorption in the thick ascending limb presumably compensates for the impaired SGK1 sensitive Na^+ reabsorption in the further distal nephron (Sandulache et al., 2006). In mice lacking SGK1, compensatory increases in Na^+ reabsorption may also occur in the proximal tubule (Wulff et al., 2002), a segment in which passive Ca^+ reabsorption also follows active Na^+ reabsorption. This may explain why the renal Ca^{2+} excretion is decreased in untreated *sgk1*$^{-/-}$ mice despite decreased TRPV5 abundance in the distal nephron (Sandulache et al., 2006). The Ca^+ channel TRPV5 mediates active Ca^{2+} reabsorption across the luminal membrane of the distal convolution (Hoenderop et al., 2003). According to coexpression studies in *Xenopus* oocytes, SGK1 increases the activity of TRPV5 at least in part by enhancing the TRPV5 protein abundance in the cell membrane (Embark et al., 2004; Palmada et al., 2005).

The present study revealed that dexamethasone reverses the anticalciuria of the *sgk1*$^{-/-}$ mice and increases renal Ca^{2+} excretion in *sgk1*$^{-/-}$ but not in *sgk1*$^{+/+}$ mice. Possibly, dexamethasone-induced Na^+ reabsorption in the aldosterone-sensitive distal nephron is at least in part independent of SGK1. Accordingly, dexamethasone treatment would be followed by fluid retention, which may suppress Na^+ and thus secondarily Ca^{2+} reabsorption in the proximal tubule and/or thick ascending limb. On the other hand, SGK1

expression has been observed in thick ascending limbs and may at least in theory participate in the regulation of electrolyte transport in this nephron segment (Alvarez et al., 2003; Lang et al., 2000). Moreover, SGK1 has been implicated in the stimulating effect of glucocorticoids on Na^+/H^+-exchanger NHE3 (Yun et al., 2002), which is of primary importance for Na^+ reabsorption in the proximal tubule (Vallon et al., 2000).

The dexamethasone-induced renal Ca^{2+} loss presumably accounts for the excessive decline of plasma Ca^{2+} concentration in *sgk1*$^{-/-}$ mice. Interestingly, the differences in plasma Ca^{2+} concentration and renal Ca^{2+} excretion between the genotypes are dissipated by additional treatment with saline. Addition of high NaCl diet significantly increased renal Ca^{2+} excretion only in *sgk1*$^{+/+}$ mice. High NaCl diet is expected to decrease SGK1 expression in wild type mice and may thus dissipate the differences between genotypes.

Glucocorticoids tended to decrease plasma potassium concentration at normal salt intake, an effect blunted after saline loading in both genotypes. During salt loading plasma K^+ concentration is significantly higher in *sgk1*$^{-/-}$ mice than in *sgk1*$^{+/+}$ mice (Huang et al., 2004). SGK1 has previously been demonstrated to regulate the renal outer medullary K^+ channel ROMK1 (Yun et al., 2002), and possibly further K^+ channels important for renal K^+ elimination. Beyond that, SGK1 dependent activity of ENaC is expected to depolarize the apical cell membrane of principal cells thus favoring K^+ secretion. As a result of both, renal potassium elimination is impaired in *sgk1*$^{-/-}$ mice (Huang et al., 2004). The defective K^+ elimination becomes particularly obvious during combined dexamethasone and salt loading. Possibly, the suppressed aldosterone release during combined dexamethasone treatment and salt load abrogates the SGK1 independent stimulation of renal K^+ excretion by aldosterone.

Apparently, SGK1 does not contribute significantly to the effect of glucocorticoids on fasting plasma glucose concentrations. Glucocorticoids have previously been shown to enhance Kv1.5 channel activity in insulin secreting ß cells and thereby suppress insulin release, an effect at least partially mediated by SGK1 (Ullrich et al., 2005). Moreover, the stimulating effect of dexamethasone on intestinal electrogenic glucose transport is blunted in mice lacking functional SGK1 (Grahammer et al., 2006). Those *in vivo* observations parallel the *in vitro* stimulating effect of SGK1 on the electrogenic Na^+-glucose cotransporter SGLT1 (Dieter et al., 2004). On the other hand SGK1 upregulates the glucose transporter GLUT1 (Palmada et al., 2006), an effect presumably contributing to

peripheral glucose uptake (Boini et al., 2006). Thus, glucocorticoid-induced and SGK1-mediated effects may increase or decrease plasma glucose concentration. Glucocorticoid excess significantly increased the peak plasma glucose concentration in both genotypes during intraperitoneal glucose load and dissipated the differences between *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ mice.

Lack of SGK1 attenuates the hypertensive effects of glucocorticoids, an observation expected, if glucocorticoid-induced salt reabsorption along the nephron is mediated in part by SGK1. In addition, the effect may reflect a role of SGK1 in the regulation of peripheral resistance. Addition of high salt diet to dexamethasone treatment blunted the difference in blood pressure between genotypes. This resembles the similar hypertensive response in *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ mice to the mineralocorticoid DOCA plus high salt diet (Vallon et al., 2005; Vallon et al., 2006). The data indicate that the SGK1-independent upregulation of renal salt reabsorption by dexamethasone is apparently sufficient to induce net renal salt retention and thus to increase blood pressure. Possibly, in both *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ mice, highly efficient pressure-natriuresis may have prevented any further acute increases in blood pressure during combined dexamethasone/high salt diet.

V. 4. Study 4: Role of SGK1 in salt sensitivity of glucose tolerance and peripheral glucose uptake

Excessive salt intake increases blood pressure, an effect paralleled by insulin resistance and/or glucose intolerance in salt sensitive subjects (Giner et al., 2001; Ogihara et al., 2003). 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. 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, i.e. 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 (Blazer-Yost et al., 2003; Blazer-Yost et al., 2004; Tong et al., 2004) but similarly affects glucose transport.

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 (Foran et al., 1999). Particularly, PKB/Akt 2 plays a key role in the stimulating effects of insulin on cellular glucose uptake (Bae et al., 2003; jiang et al., 2003; Kohn et al., 1996; McCurdy et al., 2005). Recently, PKB/Akt2 has been shown to be required for the upregulation of skeletal muscle glucose transport during calorie restriction (McCurdy et al., 2005). However, cellular glucose uptake is not disrupted in those 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 up-regulated by a variety of hormones, mediators and other regulators (Lang et al., 2001). Most importantly, SGK1 is under strong transcriptional control of mineralocorticoids (Bhargava et al., 2001; Brennan and Fuller 2000; Chen et al., 1999; Djelidi et al., 2001; Loffing et al., 2001; Pearce 2003; Shigaev et al., 2000; Verrey et al., 2003; Naray-Fejes-Toth et al., 1999). Moreover, SGK1 expression is markedly upregulated by cerebral ischaemia (Nishida et al., 2004) and may contribute to the enhancement of cellular glucose uptake in ischaemic tissues. Along those lines the enhanced cerebral SGK1 expression in enriched environment (Lee et al., 2003) may serve to adjust glucose uptake to the enhanced demand following stimulation of neuronal activity. As suggested in Fig. 26, the tissues with SGK1 sensitive glucose uptake may include the brain. Considering the virtually ubiquitous expression of SGK1 (Waldegger et al., 1997) 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 (Farjah et al., 2003), an effect at least partially mediated by a decrease of aldosterone concentration (Bhargava et al., 2001; Brennan and Fuller 2000; Chen et al., 1999; Djelidi et al., 2001; Loffing et al., 2001; Pearce 2003; Shigaev et al., 2000; Verrey et al., 2003; Naray-Fejes-Toth et al., 1999). 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 HEK223 cells, indeed confirming the ability of SGK1 to stimulate glucose transport. Most recent *in vitro* studies revealed the ability of SGK1 to enhance phloretin sensitive glucose uptake into adipocytes (Palmada et al., 2006).

The stimulating effect of SGK1 on glucose uptake into fat tissue would be expected to favour 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 (Dieter et al., 2004). 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 (Busjahn et al., 2002) presumably due to the salt retaining property of SGK1 (Wulff et al., 2002). The salt excess would again favour 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 (Roth et al., 2002). Along those lines, metabolic syndrome shares several features with glucocorticoid excess (Bahr et al., 2002) which should upregulate SGK1 expression (Firestone et al., 2003). 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 (Ullrich et al., 2005). 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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VII. PUBLICATIONS DERIVED FROM THE WORK FOR DOCTORAL THESIS

Original Publications:

- Huang DY*, **Boini KM***, Osswald H, Friedrich B, Artunc F, Ullrich S, Rajamanickam J, Palmada M, Wulff P, Kuhl D, Vallon V, Lang F: Resistance of mice lacking the serum and glucocorticoid inducible kinase SGK1 against salt-sensitive hypertension induced by high fat diet. ***Am J Physiol – Renal Physiol***, in press 2006a **(* Shared first author ship)**

- Huang DY*, **Boini KM***, Friedrich B, Metzger M, Just L, Osswald H, Wulff P, Kuhl D, Vallon V, Lang F: 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: R935-944, 2006b **(* Shared first author ship).**

- **Boini KM,** Nammi S, Grahammer F, Osswald H, Kuhl D, Vallon V, Lang F: Role of glucocorticoid inducible kinase SGK1 in glucocorticoid regulation of electrolyte excretion and blood pressure. ***Am J Physiol- Regul Integr Comp Physiol***, 2006c (Submitted).

- **Boini KM,** Hennige AM, Huang DY, Friedrich B, Palmada M, Boehmer C, Grahammer F, Artunc F, Ullrich S, Avram D, Osswald H, Wulff P, Kuhl D, Vallon V, Haering HJ, Lang F: The serum and glucocorticoid inducible kinase SGK1 mediates salt sensitivity of glucose tolerance. ***Diabetes***, 55: 2059-2066, 2006d.

Abstracts:

- Dan Y. Huang, **Krishna M. Boini**, Anita M. Hennige, Peer Wulff, Dietmer Kuhl, Florian Lang, Volker Vallon. High fat diet induced insulin resistance with salt-sensitivity of arterial blood pressure: Dependence on intact Sgk1. ***Journal of American Society of Nephrology,*** 15: 197A, 2004.
- **Krishna M. Boini,** Dan Yang Huang, Björn Friedrich, Hartmut Osswald, Peer Wulff, Dietmar Kuhl, Volker Vallon, Florian Lang. High fructose diet sensitizes blood pressure to salt intake: Resistance of mice lacking the serum and glucocorticoid inducible kinase SGK1. ***Journal of American Society of Nephrology,*** 16: 617A, 2005.
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- Dan Yang Huang, **Krishna M. Boini,** Florian Lang, Hartmut Osswald. The serum and glucocorticoid inducible kinase SGK1 mediates acute antinatriuretic effect of insulin: Insights from the knockout mice. ***Naunyn – Schmiedeberg's Archives of Pharmacology*** 369: 349, 2006.

Lebenslauf

Krishna Murthy Boini

17.08.1975	geborn in Ambala, Andhra Pradesh, Indien

Schulbildung

07/1980 – 03/1985	Grundschule in Ambala, Andhra Pradesh, Indien
06/1986 – 04/1991	Mittelschule in Warangal, Andhra Pradesh, Indien
12/1991 – 04/1994	Diplom in Pharmazie in Kurnool, Andhra Pradesh, Indien

Hochschulbildung

02/1995 – 12/1999	Bachelor of Pharmazie im Institut für Pharmazie, Andhra Universität, Visakhapatnam, India
07/2000 – 07/2002	Master of Pharmazie (Pharmakologie) im Institut für Pharmazie, Andhra Universität, Visakhapatnam, Indien unter der Anleitung von Dr. A. Annapurna **Titel**: Studie der hypoglykämikum und antihyperglykämikum Wirkung zweier pflanzlicher Rezepturen (RVF1 and RVF2) – Verabreichung der Rezepturen kombiniert und einzeln an Albinoratten.
08/2002–12/2003	Wissenschaftliche Mitarbeiter im Institut für Pharmazie, Andhra Universität, Visakhapatnam, Indien unter der Anleitung von Prof. Dr. S. Satyanarayana **Titel:** Entwicklung und Auswertung von „antistress“ Rezepturen aus ayurvedischen Pflanzenextrakten für die Behandlung von Asthma, Diabetes, Lähmungen, Bluthochdrucks und der Hautkrankheiten.
01/2004 – 2006	Anfertigung der Doktorarbeit am Institut für Physiologie, Eberhard-Karls-Universität – Tübingen, Germany unter der Anleitung von Prof. Dr. med. Florian Lang **Titel:** Rolle von SGK1 in der Salzempfindlichkeit von Blutdruck und der Glukoseaufnahme: Studien in Knockoutmäusen.