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Original Paper

Sgk1-Dependent Stimulation of Cardiac Na^+/H^+ Exchanger Nhe1 by Dexamethasone

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Key Words
Nhe1 • Na^+/H^+ exchanger • PI3K • Sgk1 • Heart failure • Dexamethasone • Glucocorticoids • HL-1 cardiomyocytes

Abstract
Background/Aims: The serum- and glucocorticoid-inducible kinase Sgk1 contributes to cardiac remodeling and development of heart failure, which is paralelled by Sgk1-dependent stimulation of the cardiac Na^+/H^+ exchanger Nhe1. Glucocorticoids are powerful stimulators of Sgk1 expression and influence cardiac remodeling. The present study thus explored whether the glucocorticoid receptor agonist dexamethasone influenced cardiac Sgk1 expression, as well as activity, expression and phosphorylation at Ser^{703} of the cardiac Na^+/H^+ exchanger Nhe1. ***Methods:*** Experiments were performed in HL-1 cardiomyocytes and gene targeted mice lacking functional Sgk1 (*sgk1*$^{-/-}$) and respective wild type mice (*sgk1*$^{+/+}$). Gene expression was determined by quantitative RT-PCR and Nhe1 phosphorylation was determined utilizing a specific antibody against a 14-3-3 binding motif at P-Ser^{703}, which represents a putative phosphorylation site recognition motif for Sgk1 and is involved in Nhe1 activation. Cytosolic pH (pH_i) was determined utilizing 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF) fluorescence and Nhe activity by the Na^+-dependent realkalinization after an ammonium pulse. ***Results:*** Ttreatment of HL-1 cardiomyocytes with dexamethasone was followed by a significant increase in *Sgk1* mRNA expression, parallelled by increased Na^+/H^+ exchanger activity. Furthermore, dexamethasone significantly increased *Nhe1* and *Spp1* mRNA expression. The effects of dexamethasone were blunted by cotreatment of HL-1 cardiomyocytes with the Sgk1 inhibitor EMD638683. Cotreatment with Nhe1 inhibitor cariporide similarly prevented dexamethasone-stimulated *Spp1* mRNA expression. In *sgk1*$^{+/+}$ mice, dexamethasone significantly increased cardiac *Sgk1* mRNA levels. In *sgk1*$^{+/+}$ mice, but not in *sgk1*$^{-/-}$ mice, dexamethasone significantly increased cardiac *Nhe1* mRNA expression and Nhe1 phosphorylation at Ser^{703}. Furthermore, cardiac *Spp1*, *Ctgf*, *Nppa* and *Nppb* mRNA levels were significantly increased in dexamethasone treated *sgk1*$^{+/+}$ mice, effects significantly blunted in *sgk1*$^{-/-}$ mice. ***Conclusions:*** Sgk1 is critically involved in the phosphorylation and activation of the cardiac Na^+/H^+ exchanger Nhe1.

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Introduction

Increased glucocorticoid levels are independent predictors of mortality in heart failure patients [1] and oral glucocorticoids are a risk factor for heart failure [2]. Along those lines treatment of preterm infants with dexamethasone is associated with hypertrophic cardiomyopathy and cardiac failure [3]. Similar observations were made in animal studies. In sheep, prenatal dexamethasone exposure leads to impaired cardiac function and cardiac hypertrophy [4]. Comparable effects were observed in rats following dexamethasone treatment [5]. In the adult rat, high doses of dexamethasone induce cardiac hypertrophy, remodeling and failure [6]. Dexamethasone further stimulates cardiac connective tissue growth factor (Ctgf) expression [7].

An early response gene strongly upregulated by glucocorticoids is the serum- and glucocorticoid-inducible kinase Sgk1 [8, 9]. Sgk1 is further stimulated by cardiac pressure overload [10-12] and genomically upregulated by a variety of further hormones [13] including mineralocorticoids [14-16], 1,25-dyhydroxyvitamin D_3 ($1,25(OH)_2D_3$) [17], gonadotropins [18-21] and TGFβ [22, 23]. The expressed kinase is activated by Phosphoinositide 3-kinase (PI3K) through phosphoinositide-dependent kinase PDK1 [24-31] and by the mammalian target of rapamycin TORC2 protein complex [32]. SGK1 abundance is particularly high in fibrosing tissue [22, 23, 33, 34]. SGK1 upregulates a wide variety of transporters [8, 9, 35, 36] including the Na^+/H^+ exchangers Nhe1 [11] and Nhe3 [37-40].

Altered expression of Sgk1 is associated with cardiac remodeling [10-12]. Lack of Sgk1 reduced the cardiac remodeling induced by pressure overload [11, 12]. Similarly, lack of Sgk1 significantly blunted the cardiac hypertrophy and fibrosis by mineralocorticoid excess [34] and angiotensin II infusion [41]. Sgk1 up-regulates Nhe1 activity [11], a key factor in cardiac remodeling and heart failure [11, 42]. Moreover, Sgk1 is required for the full transcriptional upregulation of Osteopontin (Spp1) following cardiac pressure overload [11]. Spp1 has previously been shown to be upregulated following the cardiac expression of activated Nhe1 [43].

Cardiomyocytes express the glucocorticoid receptor, and glucocorticoids also bind and activate the cardiac mineralocorticoid receptor [44, 45]. The present study thus explored whether the glucocorticoid receptor agonist dexamethasone upregulates cardiac Sgk1 transcription and whether this effect is followed by Sgk1-dependent changes in cardiac stress signalling. To this end, experiments were performed in HL-1 cardiomyocytes as well as in gene targeted mice lacking functional Sgk1 (*sgk1*$^{-/-}$) [46] and wild type control mice (*sgk1*$^{+/+}$).

Materials and Methods

Cell culture

HL-1 cardiomyocytes (kindly provided by Dr. W.C. Claycomb, Department of Biochemistry and Molecular Biology, Louisiana State University, USA) were maintained in Claycomb medium (Sigma) supplemented with 10% FBS (Sigma), 0.1 mM norepinephrine (Sigma), 2 mM L-Glutamine (Sigma), 100 units/ml penicillin and 100 µg/ml streptomycin (Invitrogen). The medium was changed approximately every 24 hours. HL-1 cardiomyocytes were seeded onto 0.02% gelatin/0.00125% fibronectin-coated dishes and cultured in normal growing medium. HL-1 cardiomyocytes were cultured in serum-free DMEM containing 1 g/l glucose (Invitrogen), supplemented with 100 units/ml penicillin and 100 µg/ml streptomycin (Invitrogen) for 4 hours prior to treatment with 500 nM dexamethasone (Sigma) and 50 µM EMD638683 [47] or 1µM cariporide [48] for 24 hours.

Intracellular pH measurements

For digital imaging of cytosolic pH (pH_i), the cells were incubated in a HEPES-buffered Ringer solution containing 10 µM BCECF-AM (Molecular Probes, Leiden, Netherlands) for 15 min at 37°C [49]. 4-6 slides per group from three independent experiments were imaged and analysed. After loading, the chamber was

flushed for 5 min with Ringer solution to remove any deesterified dye. The perfusion chamber was mounted on the stage of an inverted microscope (Zeiss Axiovert 135), which was used in the epifluorescence mode with a 40x oil immersion objective (Zeiss Neoplan, Germany). BCECF was successively excited at 490/10 and 440/10 nm, and the resultant fluorescent signal was monitored at 535/10 nm using an intensified charge-coupled device camera (Proxitronic, Germany) and specialized computer software (Metafluor, USA) [50]. Cells were outlined and monitored during the course of the measurements. Intensity ratio (490/440) data were converted into pH_i values using the high-K^+/nigericin calibration technique [51]. To this end, the cells were perfused at the end of each experiment for 5 minutes with standard high-K^+/nigericin (10 µg/ml) solution (pH 7.0). The intensity ratio data thus obtained were converted into pH values using the r_{max}, r_{min}, pK_a values previously generated from calibration experiments to generate a standard nonlinear curve (pH range 5 to 8.5) [52].

For acid loading, cells were transiently exposed to a solution containing 20 mM NH_4Cl leading to initial alkalinization of cytosolic pH (pH_i) due to entry of NH_3 and binding of H^+ to form NH_4^+ [53]. The acidification of cytosolic pH upon removal of ammonia allowed calculating the mean intrinsic buffering power (ß) of the cells [53]. Assuming that NH_4^+ and NH_3 are in equilibrium in cytosolic and extracellular fluid and that ammonia leaves the cells as NH_3:

$ß = \Delta[NH_4^+]_i/\Delta pH_i$,

where ΔpH_i is the decrease of cytosolic pH (pH_i) following ammonia removal and $\Delta[NH_4^+]_i$ is the decrease of cytosolic NH_4^+ concentration, which is identical to the concentration of $[NH_4^+]_i$ immediately before the removal of ammonia. The pK for NH_4^+/NH_3 is 8.9 [54] and at an extracellular pH (pH_o) of 7.4 the NH_4^+ concentration in extracellular fluid ($[NH_4^+]_o$) is 19.37 [$20/(1+10^{pHo-pK})$]. The intracellular NH_4^+ concentration ($[NH_4]_i$) was calculated from: $[NH_4]_i = 19.37 \cdot 10^{pHo-pHi}$ [55].

The calculation of the buffer capacity required that NH_4^+ exits completely. After the initial decline, pH_i indeed showed little further change in the absence of Na^+, indicating that there was no relevant further exit of NH_4^+. To calculate the ΔpH/min during re-alkalinization, a manual linear fit was placed over a narrow pH range (pH 6.7 to 6.9) which could be applied to the measured cells.

The solutions were composed of (in mM): standard HEPES: 115 NaCl, 5 KCl, 1 $CaCl_2$, 1.2 $MgSO_4$, 2 NaH_2PO_4 10 glucose, 32.2 HEPES; sodium free HEPES: 132.8 NMDG Cl, 3 KCl, 1 $CaCl_2$, 1.2 $MgSO_4$, 2 KH_2PO_4, 32.2 HEPES, 10 mannitol, 10 glucose (for sodium free ammonium chloride 10 mM NMDG and mannitol were replaced with 20 mM NH_4Cl); high K^+ for calibration 105 KCl, 1 $CaCl_2$, 1.2 $MgSO_4$, 32.2 HEPES, 10 mannitol, 10 µg/ml nigericin. Where indicated, 50 µM EMD638683 was added to the solutions. The pH of the solutions was titrated to 7.4 or 7.0 with HCl/NaOH, HCl/NMDG and HCl/KOH, respectively, at 37°C.

Quantitative RT-PCR

Total RNA was isolated using Trifast Reagent (Peqlab, Erlangen, Germany) according to the manufacturer's instructions. Reverse transcription of 2 µg RNA was performed using oligo$(dT)_{12-18}$ primers (Invitrogen, Karlsruhe, Germany) and SuperScript III Reverse Transcriptase (Invitrogen, Karlsruhe, Germany). cDNA samples were treated with RNase H (Invitrogen, Karlsruhe, Germany). Quantitative real-time PCR was performed with the iCycler iQ™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA) and iQ Sybr Green Supermix (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions. The following primers were used (5'→3' orientation):

Ctgf fw: GACCCAACTATGATGCGAGCC;
Ctgf rev: TCCCACAGGTCTTAGAACAGG;
Gapdh fw: AGGTCGGTGTGAACGGATTTG;
Gapdh rev: TGTAGACCATGTAGTTGAGGTCA;
Nhe1 fw: GCCATTGAGCTGGTGGAGAG;
Nhe1 rev: CGGTCTGAAGTCACAGCCTTG;
Sgk1 fw: CTGCTCGAAGCACCCTTACC;
Sgk1 rev: TCCTGAGGATGGGACATTTTCA;
Spp1 fw: GACCATGAGATTGGCAGTGA;
Spp1 rev: GGAACTGTGTTTTTGCCTCTT.

The specificity of the PCR products was confirmed by analysis of the melting curves and in addition by agarose gel electrophoresis.

To determine Anp (Nppa) and Bnp (Nppb) transcript levels, quantitative real-time PCR was performed with the iCycler iQ™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA) using Universal TaqMan Master Mix (Applied Biosystems, Foster City, CA, USA) as recommended by the manufacturer. TaqMan primers and probes for Nppa, Nppb and Gapdh were purchased from Applied Biosystems (Applied Biosystems, Foster City, CA, USA). All PCRs were performed in duplicate, and mRNA fold changes were calculated by the $2^{-\Delta\Delta Ct}$ method using Gapdh as internal reference.

Animals

All animal experiments were conducted according to the German law for the welfare of animals and were approved by local authorities. The origin of the mice has been described previously [46]. The animals were genotyped by PCR using standard methods. Prior to the experiments, mice had free access to standard rodent diet and tap drinking water. Where indicated, dexamethasone (20mg/kg BW) [56] or 0.9% NaCl as control were injected into the peritoneal cavity and 6 hours later the animals were sacrificed and cardiac tissue was immediately snap frozen in liquid nitrogen for further experiments.

Immunoprecipitation and western blotting

Nhe1 phosphorylation was estimated in Nhe1 immunoprecipitated samples using an anti-Phospho-(Ser) 14-3-3 protein binding motif antibody [57, 58]. Mouse hearts were lysed with ice-cold IP lysis buffer (Thermo Fisher Scientific, Rockford, IL, USA) supplemented with complete protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific, Rockford, IL, USA). After centrifugation at 10000 rpm for 5 min, protein concentration was determined by the Bradford assay (Biorad Laboratories, Hercules, CA). To pre-clear the lysate, fixed amounts of protein (600 µg) were incubated with control agarose resine (Thermo Fisher Scientific, Rockford, IL, USA) for 1 hour at 4 ºC. The pre-cleared lysate was incubated with 4 µl of rabbit polyclonal Nhe1 antibody (Abcam, Cambridge, UK) for 3 hours at 4 ºC. Then, immune complexes were mixed with protein A/G Agarose (Thermo Fisher Scientific, Rockford, IL, USA) for 3 hours at 4 °C and washed three times with ice-cold IP lysis buffer. The immune complexes were dissociated by addition of Roti-Load1 Buffer (Carl Roth GmbH, Karlsruhe, Germany) and heating for 5 min at 95°C. Proteins were separated on 8% SDS-polyacrylamide gels and transferred to PVDF membranes. The membranes were incubated overnight at 4°C with rabbit polyclonal Phospho-(Ser) 14-3-3 binding-motif protein antibody (Cell Signaling, Danvers, MA, USA) and then with Clean Blot IP detection reagent (Thermo Fisher Scientific, Rockford, IL, USA) for 1 hour at room temperature. Antibody binding was detected with the ECL detection reagent (Amersham, Freiburg, Germany) and bands were quantified using Quantity One Software (Bio-Rad, München, Germany). After stripping with stripping buffer (Carl Roth GmbH, Karlsruhe, Germany), the same membrane was re-probed with rabbit polyclonal Nhe1 antibody (Abcam, Cambridge, UK), and results are shown as the ratio of phosphorylated to total protein.

Statistics

Data are provided as means ± SEM, *n* represents the number of independent experiments. All data were tested for significance using Student's unpaired two-tailed t-test or ANOVA followed by post hoc analysis and only results with $p < 0.05$ were considered statistically significant. Clustal W2 software was used for multiple protein sequence alignment.

Results

In a first series of experiments, the influence of Sgk1 and dexamethasone on Na^+/H^+ exchanger activity was measured in HL-1 cardiomyocytes (Fig. 1, Table 1). Dexamethasone treatment for 24 hours upregulated the Na^+/H^+ exchanger activity in HL-1 cardiomyocytes, an effect significantly blunted by addition of 50 µM of the Sgk1 inhibitor EMD638683.

Treatment of HL-1 cardiomyocytes with 500 nM dexamethasone within 24 hours further significantly increased cardiac *Sgk1* mRNA levels. The effects of dexamethasone on *Sgk1* mRNA expression were not significantly modified by cotreatment with 50 µM of the Sgk1 inhibitor EMD638683 (Fig. 2A). As Sgk1 is known to affect *Nhe1* mRNA expression, an additional series of experiments was performed in order to test whether dexamethasone

	pH_i	Buffer capacity (mM/pH_i)	Na^+-independent $\Delta pH_i/min$	Na^+-dependent $\Delta pH_i/min$
Control	7.37 ± 0.10	8.33 ± 2.07	-0.037 ± 0.009	0.12 ± 0.03
Dexamethasone	7.40 ± 0.04	8.13 ± 1.01	-0.075 ± 0.014	0.25 ± 0.04 #
Dexamethasone +EMD638683	7.48 ± 0.07	6.66 ± 1.89	-0.066 ± 0.032	0.06 ± 0.01 **

Table 1. Cytosolic pH (pH_i), buffer capacity (mM/pH_i unit), Na^+-independent and Na^+-dependent pH_i recovery ($\Delta pH_i/min$) in HL-1 cardiomyocytes treated for 24 hours with vehicle, with 500 nM dexamethasone, or 500 nM dexamethasone and 50 μM Sgk1 inhibitor EMD638683. #($p<0.05$) indicates statistically significant difference from control treated HL-1 cardiomyocytes; **($p<0.01$) indicates statistically significant difference from dexamethasone treated HL-1 cardiomyocytes

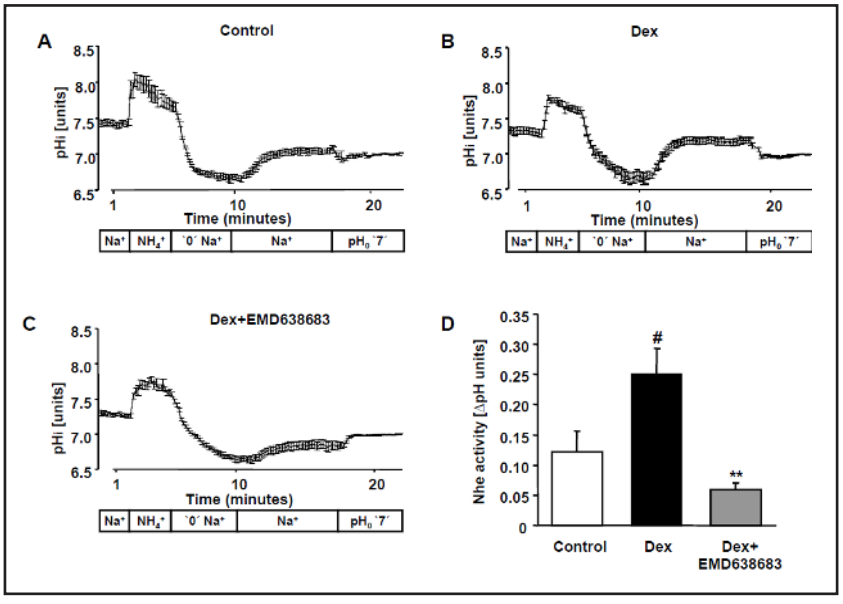

Fig. 1. Nhe activity in HL-1 cardiomyocytes following treatment with dexamethasone and Sgk inhibitor EMD638683. Time-dependent changes ± SEM of cytosolic pH (pH_i) in typical experiments in HL-1 cardiomyocytes treated for 24 hours with vehicle (A), 500 nM dexamethasone (B) or 500 nM dexamethasone and 50 μM Sgk inhibitor EMD638683 (C) following an ammonium pulse. To load the cells with H^+, 20 mM NH_4Cl was added and Na^+ removed (replaced by NMDG) in a first step (see bars below each original tracing), NH_4Cl removed in a second step, Na^+ added in a third step and nigericin (pH 7.0) applied in a fourth step to calibrate each individual experiment. (D) Arithmetic mean ± SEM (4-6 preparations/group from n=3 independent experiments) of Na^+-dependent recovery of cytosolic pH (ΔpH/min) following an ammonium pulse in HL-1 cardiomyocytes treated for 24 hours with vehicle (Control, white bar), with 500 nM dexamethasone (Dex, black bar) or with 500 nM dexamethasone and 50 μM Sgk inhibitor EMD638683 (Dex + EMD638683, grey bar) . #($p<0.05$), indicates statistically significant difference from control treated HL-1 cardiomyocytes; **($p<0.01$) indicates statistically significant difference from dexamethasone treated HL-1 cardiomyocytes.

similarly increases the *Nhe1* mRNA levels, and whether the effect requires the presence of Sgk1. As ilustrated in Fig. 2B, dexamethasone treatment of HL-1 cardiomyocytes was followed by a slight but significant increase in *Nhe1* mRNA expression, an effect blunted by cotreatment with EMD638683. In addition, the expression of the Nhe1 target Spp1 was significantly increased following dexamethasone treatment, an effect again significantly blunted by cotreatment with EMD638683 (Fig. 2C). Co-treatment with the Nhe1 inhibitor cariporide (1μM) significantly reduced the effects of dexamethasone on *Spp1* mRNA expression (Fig. 2D), an observation underscoring the role of Nhe1 in the upregulation of Spp1.

To investigate the *in vivo* relevance of Sgk1 in the regulation of Na^+/H^+ exchanger and Spp1, experiments were performed in Sgk1 knockout mice (*sgk1*$^{-/-}$) and respective wild type mice (*sgk1*$^{+/+}$). As illustrated in Fig. 3A, the levels of cardiac mRNA encoding *Sgk1* were significantly increased following the intra-peritoneal injection of dexamethasone (20 mg/kg

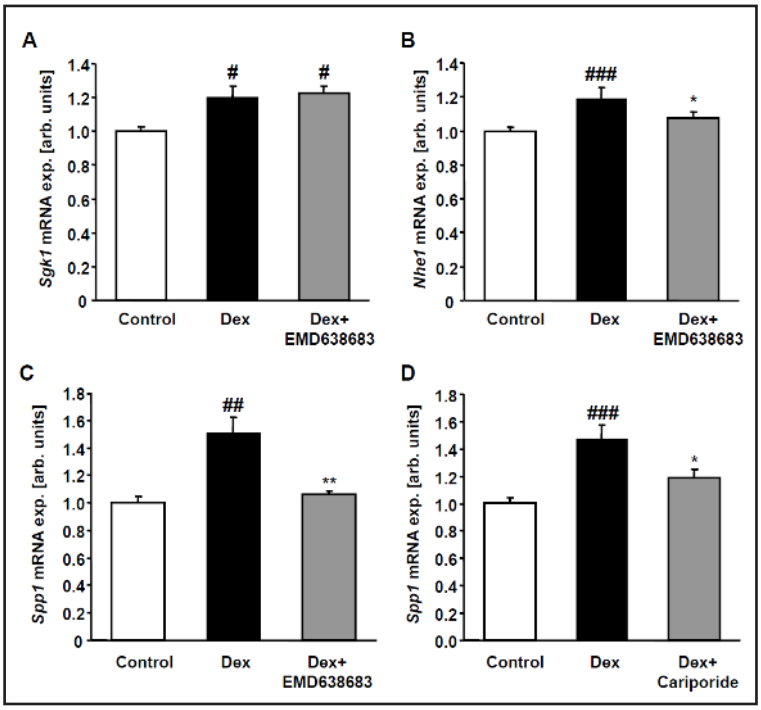

Fig. 2. Effect of dexamethasone and Sgk inhibitor EMD638683 treatment on *Sgk1*, *Nhe1* and Nhe1 target *Spp1* mRNA expression in HL-1 cardiomyocytes. Arithmetic means ± SEM (n = 4/group) of *Sgk1* (A), *Nhe1* (B) and *Spp1* (C) mRNA expression in HL-1 cardiomyocytes after 24 hours treatment with vehicle (Control, white bar), with 500 nM dexamethasone (Dex, black bar) or with 500 nM dexamethasone and 50 µM EMD638683 (Dex + EMD638683, grey bar). (D) Arithmetic means ± SEM (n = 6/group) of *Spp1* mRNA expression in HL-1 cardiomyocytes after 24 hours treatment with vehicle (Control, white bar), with 500 nM dexamethasone (Dex, black bar) or with 500 nM dexamethasone and 1 µM Nhe1 inhibitor cariporide (Dex + Cariporide, grey bar). #(p<0.05), ##(p<0.01), ###(p<0.001) indicates statistically significant difference from control treated HL-1 cardiomyocytes; *(p<0.05), **(p<0.01) indicates statistically significant difference from dexamethasone treated HL-1 cardiomyocytes.

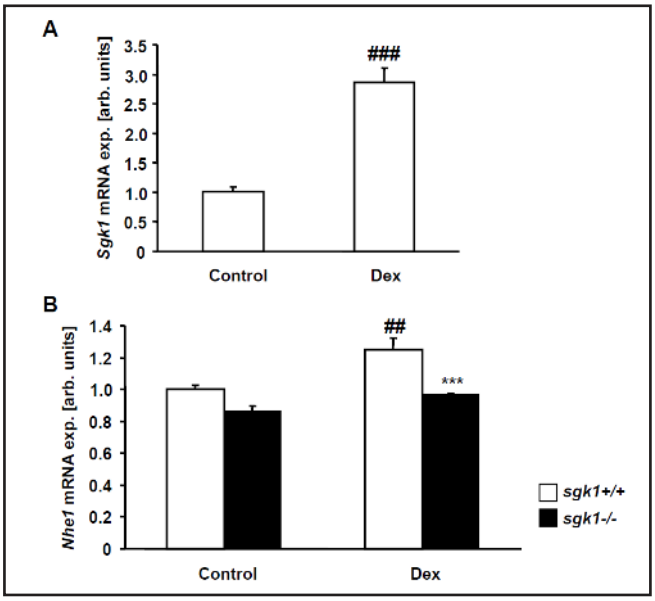

Fig. 3. Effect of dexamethasone treatment on cardiac *Sgk1* and *Nhe1* gene expression in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice. Arithmetic means ± SEM (n = 6/group) of *Sgk1* (A) and *Nhe1* (B) mRNA levels in cardiac tissue from Sgk1 knockout mice (*sgk1*$^{-/-}$, black bars) and respective wild type mice (*sgk1*$^{+/+}$, white bars) following treatment with 0.9% NaCl (0.9% NaCl, control, left bars) or with dexamethasone (20mg/kg bw, Dex, right bars). ##(p<0.01), ###(p<0.001) indicates statistically significant difference from respective control treated mice; ***(p<0.001) indicates statistically significant difference from *sgk1*$^{+/+}$ mice treated with dexamethasone.

bw). *Nhe1* mRNA expression was lower in cardiac tissue from untreated *sgk1*$^{-/-}$ mice, than in cardiac tissue from untreated *sgk1*$^{+/+}$ mice (t-test p<0.01). Dexamethasone treatment was followed by a significant increase of *Nhe1* mRNA levels in cardiac tissue from *sgk1*$^{+/+}$ mice. Accordingly, following dexamethasone treatment, the *Nhe1* mRNA expression was significantly higher in cardiac tissue from *sgk1*$^{+/+}$ mice than in cardiac tissue from *sgk1*$^{-/-}$ mice (Fig. 3B).

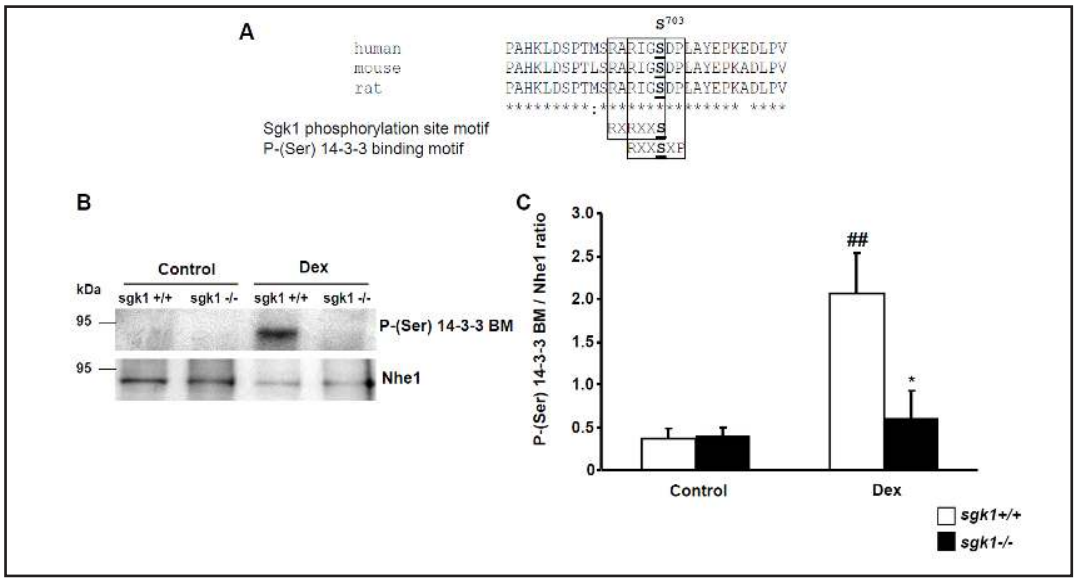

Fig. 4. Nhe1 phosphorylation at Ser[703] following dexamethasone treatment in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice. (A) Alignment of peptide sequences from Nhe1 protein around Ser[703] residue from different species. The consensus sequence for 14-3-3 binding motif to Nhe1 (RXXpSXP) around phosphorylated Ser[703] is conserved in all animal species shown. Ser[703] residue represents a putative Sgk1 phosphorylation site recognition motif (RXRXXS) and is conserved in all species shown. In the consensus sequence motifs, pS refer to the phosphoserine residue and X refers to any amino acid residues. (B) Representative original western blots for Nhe1 phosphorylation at Ser[703] detected by a specific antibody against the P-(Ser) 14-3-3 binding motif (BM) in Nhe1 immunoprecipitated samples of cardiac tissue from Sgk1 knockout mice (*sgk1*$^{-/-}$) and respective wild type mice (*sgk1*$^{+/+}$) following treatment with control (0.9% NaCl) or dexamethasone (20 mg/kg bw; Dex) injection. (C) Arithmetic means ± SEM (n = 5/group) of Nhe1 phosphorylation at Ser[703] shown as the ratio of phosphorylated to total Nhe1 protein in cardiac tissue from Sgk1 knockout mice (*sgk1*$^{-/-}$, black bars) and respective wild type mice (*sgk1*$^{+/+}$, white bars) following treatment with 0.9% NaCl (Control, left bars) or dexamethasone (20mg/kg bw; Dex, right bars). ##($p<0.01$) indicates statistically significant difference from respective control mice; *($p<0.05$) indicates statistically significant difference from *sgk1*$^{+/+}$ mice treated with dexamethasone.

Additional experiments were performed to investigate whether dexamethasone treatment influences Nhe1 phosphorylation at Ser[703]. At Ser[703], Nhe1 bears a consensus sequence specific for binding of 14-3-3 proteins (RXXpSXP, where X refers to any amino acid, and pS represents phospho-serine residue). Phosphorylation of Ser[703] is necessary for binding of 14-3-3 protein to Nhe1, which is in turn essential for Na^+/H^+ exchanger activation [59]. The sequence of Nhe1 at Ser[703] further represents a putative consensus sequence specific for Sgk1 phosphorylation site recognition motif (RXRXXS, where X refers to any amino acid, and S represents serine residue). Thus, Sgk1 might be able to phosphorylate this serine residue (Fig. 4A). Accordingly, phosphorylation of Nhe1 at Ser[703] was investigated utilizing a 14-3-3 binding motif specific antibody [58, 60, 61]. In control treated mice, the phosphorylation of Nhe1 at Ser[703] was very low in both wild type and *sgk1*$^{-/-}$ mice (Fig. 4B,C). Phosphorylation of Nhe1 was increased following dexamethasone treatment in *sgk1*$^{+/+}$ mice. In contrast, phosphorylation of Nhe1 at Ser[703] was not significantly modified following dexamethasone treatment in cardiac tissue from *sgk1*$^{-/-}$ mice. As a result, following dexamethasone treatment, the phosphorylation of Nhe1 at Ser[703] was significantly higher in cardiac tissue from *sgk1*$^{+/+}$ mice than in cardiac tissue from *sgk1*$^{-/-}$ mice.

Further experiments addressed the impact of dexamethasone on expression of the Nhe1 target Osteopontin (Spp1). Prior to dexamethasone treatment, *Spp1* mRNA levels in cardiac

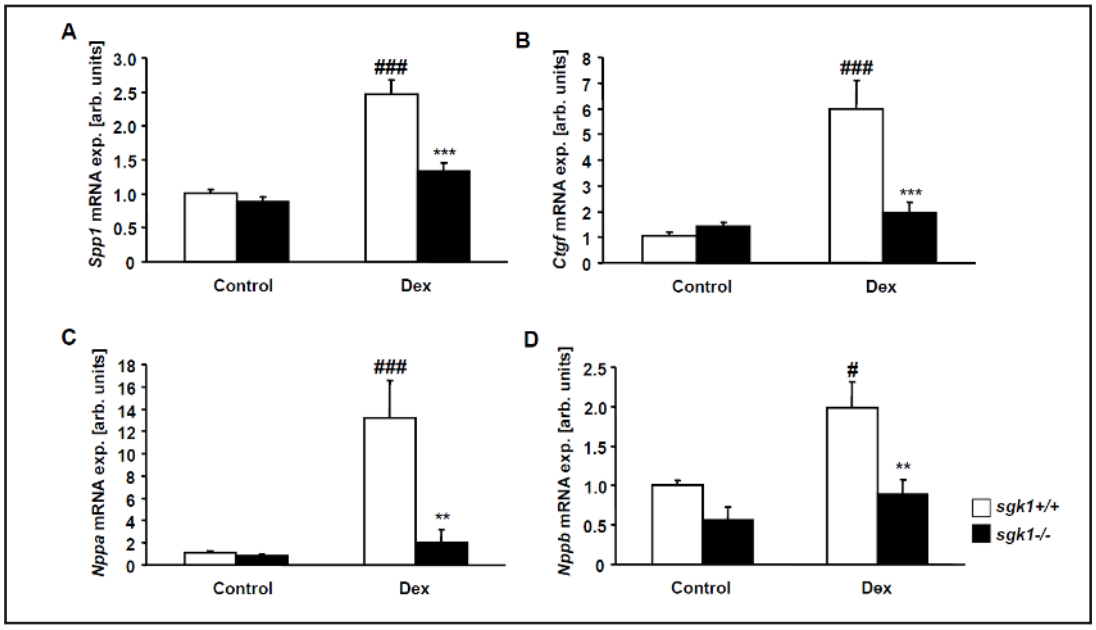

Fig. 5. Cardiac *Spp1*, *Ctgf*, *Nppa* and *Nppb* mRNA expression in $sgk1^{+/+}$ and $sgk1^{-/-}$ mice following dexamethasone treatment. Arithmetic means ± SEM (n = 6/group) of osteopontin (*Spp1*, A), connective tissue growth factor (*Ctgf*, B), atrial natriuretic peptide (*Nppa*, C) and brain natriuretic peptide (*Nppb*, D) mRNA levels in cardiac tissue from Sgk1 knockout mice ($sgk1^{-/-}$, black bars) and respective wild type mice ($sgk1^{+/+}$, white bars) following treatment with 0.9% NaCl (Control, left bars) or dexamethasone (20 mg/kg bw; Dex, right bars). #($p<0.05$), ###($p<0.001$) indicates statistically significant difference from respective control mice; **($p<0.01$), ***($p<0.001$) indicates statistically significant difference from $sgk1^{+/+}$ mice treated with dexamethasone.

tissue were similar in $sgk1^{-/-}$ mice and $sgk1^{+/+}$ mice (Fig. 5A). Dexamethasone treatment for 6 hours was followed by a significant increase of *Spp1* mRNA levels in cardiac tissue from $sgk1^{+/+}$ mice. Spp1 expression was significantly lower in dexamethasone treated $sgk1^{-/-}$ mice than in dexamethasone treated $sgk1^{+/+}$ mice. Similar observations were made on connective tissue growth factor (Ctgf) gene expression. *Ctgf* mRNA levels in cardiac tissue were similar in $sgk1^{-/-}$ mice and $sgk1^{+/+}$ mice (Fig. 5B). Dexamethasone treatment was followed by a significant increase in *Ctgf* mRNA levels in cardiac tissue from $sgk1^{+/+}$ mice but not from $sgk1^{-/-}$ mice. Accordingly, following dexamethasone treatment, the *Ctgf* transcript levels in cardiac tissue were significantly higher in $sgk1^{+/+}$ mice than in $sgk1^{-/-}$ mice.

Additional experiments elucidated the effects of dexamethasone treatment on the atrial natriuretic factor (*Nppa*) and brain natriuretic factor (*Nppb*) mRNA expression. Dexamethasone treatment was followed by a significant increase of both, *Nppa* and *Nppb* mRNA levels in cardiac tissue from $sgk1^{+/+}$ mice, effects significantly blunted in cardiac tissue from $sgk1^{-/-}$ mice. Accordingly, following dexamethasone treatment, both, *Nppa* and *Nppb* mRNA expression were significantly higher in cardiac tissue from $sgk1^{+/+}$ mice than from $sgk1^{-/-}$ mice (Fig. 5C,D).

Discussion

The present study reveals Sgk1-sensitive effects of the glucocorticoid receptor agonist dexamethasone on cardiac tissue. Treatment of HL-1 cardiomyocytes with dexamethasone leads to increased Sgk1 transcription, paralleled by increased Nhe1 transcription and Na^+/H^+ exchanger activity, both effects sensitive to the Sgk1 inhibitor EMD638683. In mice,

dexamethasone treatment similarly increased Sgk1 mRNA expression, an effect paralleled by an increase of Nhe1 transcript levels and an increase of Nhe1 phosphorylation at Ser^{703}. The effects were accompanied by genomic upregulation of Osteopontin (Spp1), Connective tissue growth factor (Ctgf), atrial natriuretic peptide (Nppa) and brain natriuretic peptide (Nppb). All those effects were diminished in gene-targeted mice lacking functional Sgk1 ($sgk1^{-/-}$) and thus required the presence of Sgk1.

Glucocorticoid excess exerts detrimental effects on the heart in animal models and humans [1, 3, 5, 6]. Glucocorticoids are strong stimulators of Sgk1 in various tissues [8]. Hearts from *sgk1*$^{-/-}$ mice are partially protected against cardiac remodeling following excessive cardiac workload, DOCA treatment and Angiotensin II infusion [11, 12, 34, 41]. Conversely, expression of constitutively active Sgk1 results in cardiomyocyte hypertrophy [10, 12]. However, the upregulation of Sgk1 contributes to but does not necessarily account for cardiomyocyte hypertrophy [11, 12, 62].

Cardiac tissue expresses primarily the Na^+/H^+ exchanger isoform Nhe1 [63, 64]. The cardiac Nhe1 has emerged as a key factor in myocardial remodeling [65-67]. Enhanced Nhe1 activity contributes to the pathophysiology of myocardial reperfusion injury [68], cardiac insufficiency [69] and cardiac hypertrophy [42, 43, 70-72]. Accordingly, upregulation of Nhe1 may trigger [73] and pharmacological inhibition of Nhe1 counteracts [65-67] cardiac hypertrophy and progression to heart failure. Moreover, Nhe1 activity participates in the regulation of cell volume [74, 75], which in turn influences protein synthesis and proteolysis [75, 76]. Nhe1 activity is further required for NADPase activity and thus formation of reactive oxygen species [77, 78]. Accordingly, inhibition of Nhe1 may prove beneficial in the treatment of heart failure [79-81].

The impact of Nhe1 on cardiac pathophysiology depends on its activity [43]. Nhe1 transport function increases Na^+ entry [82], increases intracellular Ca^{2+} content by decreasing the chemical Na^+ gradient for the Na^+/Ca^{2+} exchanger and promotes hypertrophic signalling [83-85]. The decisive mechanism accounting for the effect of cardiac Nhe1 on cardiac remodelling is, however, still incompletely understood [43].

As SGK1 is activated through phosphoinositide 3-kinase (PI3K) and phosphoinositide-dependent kinase PDK1 [24-30], SGK1 could contribute to PI3K signaling, which is known to participate in the triggering of cardiac hypertrophy [86-92]. PI3K signaling leads to activation of Nhe1 [93], an effect which cannot be attributed to Akt signaling [94].

Sgk1 stimulates the cardiac Na^+/H^+ exchanger [11]. Nhe1 activity has in turn been shown to upregulate Spp1 expression [43]. Along those lines, the enhanced Nhe1 phosphorylation in wild type mice by dexamethasone is paralleled by upregulation of Spp1 mRNA expression. As was shown earlier [7], dexamethasone also upregulated connective tissue growth factor Ctgf transcript levels, an effect that again required the presence of Sgk1. Previous studies revealed Sgk1-dependent upregulation of Ctgf expression following increased workload [11] and mineralocorticoid excess [34].

According to the present observations, phosphorylation of Nhe1 at Ser^{703} is strongly stimulated by dexamethasone in wild type mice but not in *sgk1*$^{-/-}$ mice. Accordingly, Sgk1 participates in the regulation of the Na^+/H^+ exchanger Nhe1 by glucocorticoids. Sgk1 presumably phosphorylates Nhe1 at Ser^{703}, stimulating 14-3-3 binding and Nhe1 activity. The sequence of Nhe1 at Ser^{703}, which was shown to be a specific consensus sequence for binding of 14-3-3, represents also a putative consensus sequence specific for Sgk1 phosphorylation site recognition motif. According to previous observations phosphorylation of Ser^{703} is necessary for binding of 14-3-3 protein to Nhe1 [59]. The interaction of 14-3-3 with Nhe1 could modulate Na^+/H^+ exchanger activity by preventing dephosphorylation and by stabilizing an active conformation of the exchanger [58-61]. Aldosterone stimulates the cardiac Nhe1 via phosphorylation at Ser^{703} involving epidermal growth factor receptor [95]. In accordance, Sgk1 is activated by the epidermal growth factor receptor [96]. Along those lines *sgk1*$^{-/-}$ mice are protected against DOCA induced cardiac injury [34]. It should be kept in mind though, that Sgk1 may participate in cardiac pathophysiology by regulating mechanisms in addition to Nhe1 activity [12].

In conclusion, glucocorticoid treatment leads to Sgk1-dependent genomic upregulation of Nhe1, phosphorylation of Nhe1 at Ser^{703}, and stimulation of Na^+/H^+ exchanger activity. Glucocorticoids trigger Sgk1-dependent cardiac stress signalling, events reminiscent of cardiac effects following mineralocorticoid excess or chronic workload. The present observations underscore the functional significance of serum- and glucocorticoid-inducible kinase 1 in cardiac pathophysiology [97].

Abbreviations

Nppa (Atrial natriuretic peptide);Nppb (Brain natriuretic peptide); Spp1 (Secreted phosphoprotein 1, Osteopontin); Ctgf (Connective tissue growth factor); Nhe (Na^+/H^+ exchanger); PI3K (Phosphoinositide 3-kinase); Sgk1 (Serum- and glucocorticoid-inducible kinase 1).

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References

1 Guder G, Bauersachs J, Frantz S, Weismann D, Allolio B, Ertl G, Angermann CE, Stork S: Complementary and incremental mortality risk prediction by cortisol and aldosterone in chronic heart failure. Circulation 2007;115:1754-1761.
2 Souverein PC, Berard A, Van Staa TP, Cooper C, Egberts AC, Leufkens HG, Walker BR: Use of oral glucocorticoids and risk of cardiovascular and cerebrovascular disease in a population based case-control study. Heart 2004;90:859-865.
3 Israel BA, Sherman FS, Guthrie RD: Hypertrophic cardiomyopathy associated with dexamethasone therapy for chronic lung disease in preterm infants. Am J Perinatol 1993;10:307-310.
4 Dodic M, Samuel C, Moritz K, Wintour EM, Morgan J, Grigg L, Wong J: Impaired cardiac functional reserve and left ventricular hypertrophy in adult sheep after prenatal dexamethasone exposure. Circ Res 2001;89:623-629.
5 de Vries WB, van der Leij FR, Bakker JM, Kamphuis PJ, van Oosterhout MF, Schipper ME, Smid GB, Bartelds B, van Bel F: Alterations in adult rat heart after neonatal dexamethasone therapy. Pediatr Res 2002;52:900-906.
6 De P, Roy SG, Kar D, Bandyopadhyay A: Excess of glucocorticoid induces myocardial remodeling and alteration of calcium signaling in cardiomyocytes. J Endocrinol 2011;209:105-114.
7 Dammeier J, Beer HD, Brauchle M, Werner S: Dexamethasone is a novel potent inducer of connective tissue growth factor expression. Implications for glucocorticoid therapy. J Biol Chem 1998;273:18185-18190.
8 Lang F, Cohen P: Regulation and physiological roles of serum- and glucocorticoid-induced protein kinase isoforms. Sci STKE 2001;2001:re17.
9 Lang F, Bohmer C, Palmada M, Seebohm G, Strutz-Seebohm N, Vallon V: (Patho)physiological significance of the serum- and glucocorticoid-inducible kinase isoforms. Physiol Rev 2006;86:1151-1178.
10 Aoyama T, Matsui T, Novikov M, Park J, Hemmings B, Rosenzweig A: Serum and glucocorticoid-responsive kinase-1 regulates cardiomyocyte survival and hypertrophic response. Circulation 2005;111:1652-1659.

11 Voelkl J, Lin Y, Alesutan I, Ahmed MS, Pasham V, Mia S, Gu S, Feger M, Saxena A, Metzler B, Kuhl D, Pichler BJ, Lang F: Sgk1 sensitivity of Na(+)/H(+) exchanger activity and cardiac remodeling following pressure overload. Basic Res Cardiol 2012;107:236.
12 Das S, Aiba T, Rosenberg M, Hessler K, Xiao C, Quintero PA, Ottaviano FG, Knight AC, Graham EL, Bostrom P, Morissette MR, del Monte F, Begley MJ, Cantley LC, Ellinor PT, Tomaselli GF, Rosenzweig A: Pathological role of serum- and glucocorticoid-regulated kinase 1 in adverse ventricular remodeling. Circulation 2012;126:2208-2219.
13 Firestone GL, Giampaolo JR, O'Keeffe BA: Stimulus-dependent regulation of serum and glucocorticoid inducible protein kinase (SGK) transcription, subcellular localization and enzymatic activity. Cell Physiol Biochem 2003;13:1-12.
14 Chen SY, Bhargava A, Mastroberardino L, Meijer OC, Wang J, Buse P, Firestone GL, Verrey F, Pearce D: Epithelial sodium channel regulated by aldosterone-induced protein sgk. Proc Natl Acad Sci U S A 1999;96:2514-2519.
15 Naray-Fejes-Toth A, Canessa C, Cleaveland ES, Aldrich G, Fejes-Toth G: Sgk is an aldosterone-induced kinase in the renal collecting duct. Effects on epithelial Na^+ channels. J Biol Chem 1999;274:16973-16978.
16 Shigaev A, Asher C, Latter H, Garty H, Reuveny E: Regulation of sgk by aldosterone and its effects on the epithelial Na^+ channel. Am J Physiol Renal Physiol 2000;278:F613-F619.
17 Akutsu N, Lin R, Bastien Y, Bestawros A, Enepekides DJ, Black MJ, White JH: Regulation of gene Expression by 1alpha,25-dihydroxyvitamin D3 and Its analog EB1089 under growth-inhibitory conditions in squamous carcinoma Cells. Mol Endocrinol 2001;15:1127-1139.
18 Alliston TN, Maiyar AC, Buse P, Firestone GL, Richards JS: Follicle stimulating hormone-regulated expression of serum/glucocorticoid-inducible kinase in rat ovarian granulosa cells: a functional role for the Sp1 family in promoter activity. Mol Endocrinol 1997;11:1934-1949.
19 Alliston TN, Gonzalez-Robayna IJ, Buse P, Firestone GL, Richards JS: Expression and localization of serum/glucocorticoid-induced kinase in the rat ovary: relation to follicular growth and differentiation. Endocrinology 2000;141:385-395.
20 Gonzalez-Robayna IJ, Falender AE, Ochsner S, Firestone GL, Richards JS: Follicle-Stimulating hormone (FSH) stimulates phosphorylation and activation of protein kinase B (PKB/Akt) and serum and glucocorticoid-lnduced kinase (Sgk): evidence for A kinase-independent signaling by FSH in granulosa cells. Mol Endocrinol 2000;14:1283-1300.
21 Richards JS, Fitzpatrick SL, Clemens JW, Morris JK, Alliston T, Sirois J: Ovarian cell differentiation: a cascade of multiple hormones, cellular signals, and regulated genes. Recent Prog Horm Res 1995;50:223-254.
22 Lang F, Klingel K, Wagner CA, Stegen C, Warntges S, Friedrich B, Lanzendorfer M, Melzig J, Moschen I, Steuer S, Waldegger S, Sauter M, Paulmichl M, Gerke V, Risler T, Gamba G, Capasso G, Kandolf R, Hebert SC, Massry SG, Broer S: Deranged transcriptional regulation of cell-volume-sensitive kinase hSGK in diabetic nephropathy. Proc Natl Acad Sci U S A 2000;97:8157-8162.
23 Waldegger S, Klingel K, Barth P, Sauter M, Rfer ML, Kandolf R, Lang F: h-sgk serine-threonine protein kinase gene as transcriptional target of transforming growth factor beta in human intestine. Gastroenterology 1999;116:1081-1088.
24 Alessi DR, Andjelkovic M, Caudwell B, Cron P, Morrice N, Cohen P, Hemmings BA: Mechanism of activation of protein kinase B by insulin and IGF-1. EMBO J 1996;15:6541-6551.
25 Alessi DR, Cohen P: Mechanism of activation and function of protein kinase B. Curr Opin Genet Dev 1998;8:55-62.
26 Divecha N, Banfic H, Irvine RF: The polyphosphoinositide cycle exists in the nuclei of Swiss 3T3 cells under the control of a receptor (for IGF-I) in the plasma membrane, and stimulation of the cycle increases nuclear diacylglycerol and apparently induces translocation of protein kinase C to the nucleus. EMBO J 1991;10:3207-3214.
27 Gamper N, Fillon S, Huber SM, Feng Y, Kobayashi T, Cohen P, Lang F: IGF-1 up-regulates K^+ channels via PI3-kinase, PDK1 and SGK1. Pflugers Arch 2002;443:625-634.
28 Kobayashi T, Cohen P: Activation of serum- and glucocorticoid-regulated protein kinase by agonists that activate phosphatidylinositide 3-kinase is mediated by 3-phosphoinositide-dependent protein kinase-1 (PDK1) and PDK2. Biochem J 1999;339:319-328.

29 Kotani K, Yonezawa K, Hara K, Ueda H, Kitamura Y, Sakaue H, Ando A, Chavanieu A, Grigorescu F: Involvement of phosphoinositide 3-kinase in insulin- or IGF-1-induced membrane ruffling. EMBO J 1994;13:2313-2321.
30 Park J, Leong ML, Buse P, Maiyar AC, Firestone GL, Hemmings BA: Serum and glucocorticoid-inducible kinase (SGK) is a target of the PI 3-kinase-stimulated signaling pathway. EMBO J 1999;18:3024-3033.
31 Schmidt EM, Kraemer BF, Borst O, Munzer P, Schonberger T, Schmidt C, Leibrock C, Towhid ST, Seizer P, Kuhl D, Stournaras C, Lindemann S, Gawaz M, Lang F: SGK1 sensitivity of platelet migration. Cell Physiol Biochem 2012;30:259-268.
32 Lu M, Wang J, Ives HE, Pearce D: mSIN1 protein mediates SGK1 protein interaction with mTORC2 protein complex and is required for selective activation of the epithelial sodium channel. J Biol Chem 2011;286:30647-30654.
33 Feng Y, Wang Q, Wang Y, Yard B, Lang F: SGK1-mediated fibronectin formation in diabetic nephropathy. Cell Physiol Biochem 2005;16:237-244.
34 Vallon V, Wyatt AW, Klingel K, Huang DY, Hussain A, Berchtold S, Friedrich B, Grahammer F, Belaiba RS, Gorlach A, Wulff P, Daut J, Dalton ND, Ross J, Jr., Flogel U, Schrader J, Osswald H, Kandolf R, Kuhl D, Lang F: SGK1-dependent cardiac CTGF formation and fibrosis following DOCA treatment. J Mol Med 2006;84:396-404.
35 Alesutan IS, Ureche ON, Laufer J, Klaus F, Zurn A, Lindner R, Strutz-Seebohm N, Tavare JM, Boehmer C, Palmada M, Lang UE, Seebohm G, Lang F: Regulation of the glutamate transporter EAAT4 by PIKfyve. Cell Physiol Biochem 2010;25:187-194.
36 Bohmer C, Sopjani M, Klaus F, Lindner R, Laufer J, Jeyaraj S, Lang F, Palmada M: The serum and glucocorticoid inducible kinases SGK1-3 stimulate the neutral amino acid transporter SLC6A19. Cell Physiol Biochem 2010;25:723-732.
37 Fuster DG, Bobulescu IA, Zhang J, Wade J, Moe OW: Characterization of the regulation of renal Na^+/H^+ exchanger NHE3 by insulin. Am J Physiol Renal Physiol 2007;292:F577-F585.
38 He P, Lee SJ, Lin S, Seidler U, Lang F, Fejes-Toth G, Naray-Fejes-Toth A, Yun CC: Serum- and glucocorticoid-induced kinase 3 in recycling endosomes mediates acute activation of Na^+/H^+ exchanger NHE3 by glucocorticoids. Mol Biol Cell 2011;22:3812-3825.
39 Wang D, Zhang H, Lang F, Yun CC: Acute activation of NHE3 by dexamethasone correlates with activation of SGK1 and requires a functional glucocorticoid receptor. Am J Physiol Cell Physiol 2007;292:C396-C404.
40 Yun CC, Chen Y, Lang F: Glucocorticoid activation of Na^+/H^+ exchanger isoform 3 revisited. The roles of SGK1 and NHERF2. J Biol Chem 2002;277:7676-7683.
41 Yang M, Zheng J, Miao Y, Wang Y, Cui W, Guo J, Qiu S, Han Y, Jia L, Li H, Cheng J, Du J: Serum-glucocorticoid regulated kinase 1 regulates alternatively activated macrophage polarization contributing to angiotensin II-induced inflammation and cardiac fibrosis. Arterioscler Thromb Vasc Biol 2012;32:1675-1686.
42 Fliegel L: Regulation of the Na^+/H^+ exchanger in the healthy and diseased myocardium. Expert Opin Ther Targets 2009;13:55-68.
43 Xue J, Mraiche F, Zhou D, Karmazyn M, Oka T, Fliegel L, Haddad GG: Elevated myocardial Na^+/H^+ exchanger isoform 1 activity elicits gene expression that leads to cardiac hypertrophy. Physiol Genomics 2010;42:374-383.
44 Katz SE, Penefsky ZJ, McGinnis MY: Cytosolic glucocorticoid receptors in the developing rat heart. J Mol Cell Cardiol 1988;20:323-328.
45 Mihailidou AS, Loan LT, Mardini M, Funder JW: Glucocorticoids activate cardiac mineralocorticoid receptors during experimental myocardial infarction. Hypertension 2009;54:1306-1312.
46 Wulff P, Vallon V, Huang DY, Volkl H, Yu F, Richter K, Jansen M, Schlunz M, Klingel K, Loffing J, Kauselmann G, Bosl MR, Lang F, Kuhl D: Impaired renal Na^+ retention in the sgk1-knockout mouse. J Clin Invest 2002;110:1263-1268.
47 Ackermann TF, Boini KM, Beier N, Scholz W, Fuchss T, Lang F: EMD638683, a novel SGK inhibitor with antihypertensive potency. Cell Physiol Biochem 2011;28:137-146.
48 Toda T, Kadono T, Hoshiai M, Eguchi Y, Nakazawa S, Nakazawa H, Higashijima N, Ishida H: Na^+/H^+ exchanger inhibitor cariporide attenuates the mitochondrial Ca^{2+} overload and PTP opening. Am J Physiol Heart Circ Physiol 2007;293:H3517-H3523.
49 Yang W, Bhandaru M, Pasham V, Bobbala D, Zelenak C, Jilani K, Rotte A, Lang F: Effect of thymoquinone on cytosolic pH and Na+/H+ exchanger activity in mouse dendritic cells. Cell Physiol Biochem 2012;29:21-30.

50 Rotte A, Pasham V, Eichenmuller M, Yang W, Bhandaru M, Lang F: Influence of dexamethasone on Na^+/H^+ exchanger activity in dendritic cells. Cell Physiol Biochem 2011;28:305-314.
51 Waisbren SJ, Geibel J, Boron WF, Modlin IM: Luminal perfusion of isolated gastric glands. Am J Physiol 1994;266:C1013-C1027.
52 Bhandaru M, Pasham V, Yang W, Bobbala D, Rotte A, Lang F: Effect of azathioprine on Na^+/H^+ exchanger activity in dendritic cells. Cell Physiol Biochem 2012;29:533-542.
53 Roos A, Boron WF: Intracellular pH. Physiol Rev 1981;61:296-434.
54 Boyarsky G, Ganz MB, Sterzel RB, Boron WF: pH regulation in single glomerular mesangial cells. II. Na^+-dependent and -independent Cl^--. Am J Physiol 1988;255:C857-C869.
55 Rotte A, Pasham V, Bhandaru M, Bobbala D, Zelenak C, Lang F: Rapamycin sensitive ROS formation and Na^+/H^+ exchanger activity in dendritic cells. Cell Physiol Biochem 2012;29:543-550.
56 Xu B, Strom J, Chen QM: Dexamethasone induces transcriptional activation of Bcl-xL gene and inhibits cardiac injury by myocardial ischemia. Eur J Pharmacol 2011;668:194-200.
57 Cingolani OH, Perez NG, Ennis IL, Alvarez MC, Mosca SM, Schinella GR, Escudero EM, Console G, Cingolani HE: In vivo key role of reactive oxygen species and NHE-1 activation in determining excessive cardiac hypertrophy. Pflugers Arch 2011;462:733-743.
58 Yeves AM, Garciarena CD, Nolly MB, Chiappe de Cingolani GE, Cingolani HE, Ennis IL: Decreased activity of the Na^+/H^+ exchanger by phosphodiesterase 5A inhibition is attributed to an increase in protein phosphatase activity. Hypertension 2010;56:690-695.
59 Lehoux S, Abe J, Florian JA, Berk BC: 14-3-3 Binding to Na^+/H^+ exchanger isoform-1 is associated with serum-dependent activation of Na^+/H^+ exchange. J Biol Chem 2001;276:15794-15800.
60 Chen S, Mackintosh C: Differential regulation of NHE1 phosphorylation and glucose uptake by inhibitors of the ERK pathway and p90RSK in 3T3-L1 adipocytes. Cell Signal 2009;21:1984-1993.
61 Maekawa N, Abe J, Shishido T, Itoh S, Ding B, Sharma VK, Sheu SS, Blaxall BC, Berk BC: Inhibiting p90 ribosomal S6 kinase prevents Na^+/H^+ exchanger-mediated cardiac ischemia-reperfusion injury. Circulation 2006;113:2516-2523.
62 Lister K, Autelitano DJ, Jenkins A, Hannan RD, Sheppard KE: Cross talk between corticosteroids and alpha-adrenergic signalling augments cardiomyocyte hypertrophy: a possible role for SGK1. Cardiovasc Res 2006;70:555-565.
63 Karmazyn M, Gan XT, Humphreys RA, Yoshida H, Kusumoto K: The myocardial Na^+/H^+ exchange: structure, regulation, and its role in heart disease. Circ Res 1999;85:777-786.
64 Rieder CV, Fliegel L: Transcriptional regulation of Na^+/H^+ exchanger expression in the intact mouse. Mol Cell Biochem 2003;243:87-95.
65 Aker S, Snabaitis AK, Konietzka I, Van De SA, Bongler K, Avkiran M, Heusch G, Schulz R: Inhibition of the Na^+/H^+ exchanger attenuates the deterioration of ventricular function during pacing-induced heart failure in rabbits. Cardiovasc Res 2004;63:273-282.
66 Engelhardt S, Hein L, Keller U, Klambt K, Lohse MJ: Inhibition of Na^+/H^+ exchange prevents hypertrophy, fibrosis, and heart failure in beta(1)-adrenergic receptor transgenic mice. Circ Res 2002;90:814-819.
67 Leineweber K, Aker S, Beilfuss A, Rekasi H, Konietzka I, Martin C, Heusch G, Schulz R: Inhibition of Na^+/H^+-exchanger with sabiporide attenuates the downregulation and uncoupling of the myocardial beta-adrenoceptor system in failing rabbit hearts. Br J Pharmacol 2006;148:137-146.
68 Wang Y, Meyer JW, Ashraf M, Shull GE: Mice with a null mutation in the NHE1 Na^+/H^+ exchanger are resistant to cardiac ischemia-reperfusion injury. Circ Res 2003;93:776-782.
69 Yokoyama H, Gunasegaram S, Harding SE, Avkiran M: Sarcolemmal Na^+/H^+ exchanger activity and expression in human ventricular myocardium. J Am Coll Cardiol 2000;36:534-540.
70 Leineweber K, Heusch G, Schulz R: Regulation and role of the presynaptic and myocardial Na^+/H^+ exchanger NHE1: effects on the sympathetic nervous system in heart failure. Cardiovasc Drug Rev 2007;25:123-131.
71 Mraiche F, Oka T, Gan XT, Karmazyn M, Fliegel L: Activated NHE1 is required to induce early cardiac hypertrophy in mice. Basic Res Cardiol 2011;106:603-616.
72 Mraiche F, Fliegel L: Elevated expression of activated Na^+/H^+ exchanger protein induces hypertrophy in isolated rat neonatal ventricular cardiomyocytes. Mol Cell Biochem 2011;358:179-187.
73 Nakamura TY, Iwata Y, Arai Y, Komamura K, Wakabayashi S: Activation of Na^+/H^+ exchanger 1 is sufficient to generate Ca^{2+} signals that induce cardiac hypertrophy and heart failure. Circ Res 2008;103:891-899.

74 Hoffmann EK, Lambert IH, Pedersen SF: Physiology of cell volume regulation in vertebrates. Physiol Rev 2009;89:193-277.
75 Lang F, Busch GL, Ritter M, Volkl H, Waldegger S, Gulbins E, Haussinger D: Functional significance of cell volume regulatory mechanismsr. Physiol Rev 1998;78:247-306.
76 Haussinger D, Lang F: Cell volume in the regulation of hepatic function: a mechanism for metabolic control. Biochim Biophys Acta 1991;1071:331-350.
77 Cingolani HE, Ennis IL, Aiello EA, Perez NG: Role of autocrine/paracrine mechanisms in response to myocardial strain. Pflugers Arch 2011; 462: 29-38.
78 Garciarena CD, Caldiz CI, Correa MV, Schinella GR, Mosca SM, Chiappe de Cingolani GE, Cingolani HE, Ennis IL: Na^+/H^+ exchanger-1 inhibitors decrease myocardial superoxide production via direct mitochondrial action. J Appl Physiol 2008;105:1706-1713.
79 Baartscheer A: Chronic inhibition of Na^+/H^+-exchanger in the heart. Curr Vasc Pharmacol 2006;4:23-29.
80 Karmazyn M, Kilic A, Javadov S: The role of NHE-1 in myocardial hypertrophy and remodelling. J Mol Cell Cardiol 2008;44:647-653.
81 Morris K: Targeting the myocardial sodium-hydrogen exchange for treatment of heart failure. Expert Opin Ther Targets 2002;6:291-298.
82 Mraiche F, Wagg CS, Lopaschuk GD, Fliegel L: Elevated levels of activated NHE1 protect the myocardium and improve metabolism following ischemia/reperfusion injury. J Mol Cell Cardiol 2011;50:157-164.
83 Eigel BN, Hadley RW: Contribution of the Na^+ channel and Na^+/H^+ exchanger to the anoxic rise of $[Na^+]$ in ventricular myocytes. Am J Physiol 1999;277:H1817-H1822.
84 Hisamitsu T, Nakamura TY, Wakabayashi S: Na^+/H^+ exchanger 1 directly binds to calcineurin A and activates downstream NFAT signaling, leading to cardiomyocyte hypertrophy. Mol Cell Biol 2012;32:3265-3280.
85 Allen DG, Xiao XH: Role of the cardiac Na^+/H^+ exchanger during ischemia and reperfusion. Cardiovasc Res 2003;57:934-941.
86 Alvin Z, Laurence GG, Coleman BR, Zhao A, Hajj-Moussa M, Haddad GE: Regulation of L-type inward calcium channel activity by captopril and angiotensin II via the phosphatidyl inositol 3-kinase pathway in cardiomyocytes from volume-overload hypertrophied rat hearts. Can J Physiol Pharmacol 2011;89:206-215.
87 Damilano F, Perino A, Hirsch E: PI3K kinase and scaffold functions in heart. Ann N Y Acad Sci 2010;1188:39-45.
88 Gruson D, Ginion A, Decroly N, Lause P, Vanoverschelde JL, Ketelslegers JM, Bertrand L, Thissen JP: Urocortin-induced cardiomyocytes hypertrophy is associated with regulation of the GSK-3beta pathway. Heart Vessels 2012;27:202-207.
89 Guo D, Kassiri Z, Basu R, Chow FL, Kandalam V, Damilano F, Liang W, Izumo S, Hirsch E, Penninger JM, Backx PH, Oudit GY: Loss of PI3Kgamma enhances cAMP-dependent MMP remodeling of the myocardial N-cadherin adhesion complexes and extracellular matrix in response to early biomechanical stress. Circ Res 2010;107:1275-1289.
90 Oudit GY, Penninger JM: Cardiac regulation by phosphoinositide 3-kinases and PTEN. Cardiovasc Res 2009;82:250-260.
91 Rohini A, Agrawal N, Koyani CN, Singh R: Molecular targets and regulators of cardiac hypertrophy. Pharmacol Res 2010;61:269-280.
92 Yu W, Chen C, Fu Y, Wang X, Wang W: Insulin signaling: a possible pathogenesis of cardiac hypertrophy. Cardiovasc Ther 2010;28:101-105.
93 Lawrence SP, Holman GD, Koumanov F: Translocation of the Na^+/H^+ exchanger 1 (NHE1) in cardiomyocyte responses to insulin and energy-status signalling. Biochem J 2010;432:515-523.
94 Snabaitis AK, Cuello F, Avkiran M: Protein kinase B/Akt phosphorylates and inhibits the cardiac Na^+/H^+ exchanger NHE1. Circ Res 2008;103:881-890.
95 De Giusti VC, Nolly MB, Yeves AM, Caldiz CI, Villa-Abrille MC, Chiappe de Cingolani GE, Ennis IL, Cingolani HE, Aiello EA: Aldosterone stimulates the cardiac Na^+/H^+ exchanger via transactivation of the epidermal growth factor receptor. Hypertension 2011;58:912-919.
96 Stevens VA, Saad S, Chen XM, Pollock CA: The interdependence of EGF-R and SGK-1 in fibronectin expression in primary kidney cortical fibroblast cells. Int J Biochem Cell Biol 2007;39:1047-1054.
97 Lang F, Gorlach A: Heterocyclic indazole derivatives as SGK1 inhibitors, WO2008138448. Expert Opin Ther Pat 2010;20:129-135.