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 paralleled 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 Ser⁷⁰³ 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-Ser⁷⁰³, which represents a putative phosphorylation site recognition motif for Sgk1 and is involved in Nhe1 activation. Cytosolic pH (pHᵢ) 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: Treatment of HL-1 cardiomyocytes with dexamethasone was followed by a significant increase in Sgk1 mRNA expression, paralleled by increased Na⁺/H⁺ exchanger activity. Furthermore, cardiac Spp1 mRNA expression was 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.
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-dihydroxyvitamin D$_3$ (1,25(OH)$_2$D$_3$) [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 Axiosvert 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 (MetaFluo, USA) [50]. Cells were outlined and monitored during the course of the measurements. Intensity ratio (490/440) data were converted into pH 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_{med} r_{med} pK values previously generated from calibration experiments to generate a standard nonlinear curve (pH range 5 to 8.5) [52].

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 CaCl₂, 1.2 MgSO₄, 2 NaH₂PO₄, 10 glucose, 32.2 HEPES; sodium free HEPES: 132.8 NMDG Cl, 1 CaCl₂, 1.2 MgSO₄, 2 KH₂PO₄, 32.2 HEPES, 10 mannitol, 10 glucose (for sodium free ammonium chloride 10 mM NMDG and mannitol were replaced with 20 mM NH₄Cl); high K+ for calibration 105 KCl, 1 CaCl₂, 1.2 MgSO₄, 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: GACCCAACTATGATGCGACC;
- Ctgf rev: TCCCACAGTCTTGAACAGG;
- Gapdh fw: AGTCTGGTGATGAGGATTG;
- Gapdh rev: TGTTGACATGAGTTGAGTC;
- Nhe1 fw: GCCATTGAGCTGGTGGAGAG;
- Nhe1 rev: CGGTCTGAAGTCACAGCCTTG;
- Sgk1 fw: CTGCCAGAGGGCCTTACC;
- Sgk1 rev: TCTCGGATGCTGGCAGACTT;
- Spp1 fw: GACCAGATTGGCGAGTGA;
- Spp1 rev: GGCGAGTTGCCTGCTTCTT.

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^(-ΔΔCt) 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 resin (Thermo Fisher Scientific, Rockford, IL, USA) for 1 hour at 4°C. The pre-cleared lysate was incubated with 4 µl of rabbit polyclonal Nhe1 antibody (Abcam, Cambridge, UK) for 3 hours at 4°C. 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...
similarly increases the Nhe1 mRNA levels, and whether the effect requires the presence of Sgk1. As illustrated 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.

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...
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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.

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).
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 (RXXRXS, 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...
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, B) 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,
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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\textsuperscript{703}, and stimulation of Na\textsuperscript{+}/H\textsuperscript{+} 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\textsuperscript{+}/H\textsuperscript{+} exchanger); PI3K (Phosphoinositide 3-kinase); Sgk1 (Serum- and glucocorticoid-inducible kinase 1).

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