Increases in intracellular sodium activate transcription and gene expression via the salt-inducible kinase 1 network in an atrial myocyte cell line

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Am J Physiol Heart Circ Physiol 303: H57–H65, 2012. First published March 30, 2012; doi:10.1152/ajpheart.00512.2011.—Cardiac hypertrophy (CH) generally occurs as the result of the sustained mechanical stress caused by elevated systemic arterial blood pressure (BP). However, in animal models, elevated salt intake is associated with CH even in the absence of significant increases in BP. We hypothesize that CH is not exclusively the consequence of mechanical stress but also of other factors associated with elevated BP such as abnormal cell sodium homeostasis. We examined the effect of small increases in intracellular sodium concentration ([Na+]i) on transcription factors and genes associated with CH in a cardiac cell line. Increases in [Na+]i led to a time-dependent increase in the expression levels of mRNA for natriuretic peptide and myosin heavy chain genes and also increased myocyte enhancer factor (MEF2)/nuclear factor of activated T cell (NFAT) transcriptional activity. Increases in [Na+]i are associated with activation of salt-inducible kinase 1 (snflk-1, SIK1), a kinase known to be critical for cardiac development. Moreover, increases in [Na+]i resulted in increased SIK1 expression. Sodium did not increase MEF2/NFAT activity or gene expression in cells expressing a SIK1 that lacked kinase activity. The mechanism by which SIK1 activated MEF2 involved phosphorylation of HDAC5. Increases in [Na+]i activate SIK1 and MEF2 via a parallel increase in intracellular calcium through the reverse mode of Na+/Ca2+-exchanger and activation of CaMK1. These data obtained in a cardiac cell line suggest that increases in intracellular sodium could influence myocardial growth by controlling transcriptional activation and gene expression throughout the activation of the SIK1 network.

hypertension; cardiac hypertrophy; Na+,K+-ATPase; ion gradients

CARDIAC HYPERTROPHY IS CONSIDERED to be the heart’s response to sustained mechanical stress caused by either obstruction of cardiac outflow (valve stenosis) or elevated systemic arterial blood pressure. At the molecular level, its development involves the activation of numerous genes and transcription factors (9, 18, 30) that ultimately lead to abnormal myocardial growth. Hypertension is presently one of the major causes of cardiac hypertrophy. Although systemic hypertension has multiple causes, it ultimately results from the kidney’s inability to maintain sodium homeostasis after normal or excessive sodium intake (1, 16). Whereas a sustained increase in systemic blood pressure is likely to influence the development of cardiac hypertrophy, it has also been demonstrated that high salt intake is associated with left ventricle hypertrophy even without significant increases in systemic blood pressure and independently of systemic renin-angiotensin-aldosterone levels (10, 40, 41). This raises the possibility that cardiac hypertrophy may not necessarily be related exclusively to the mechanical stress imposed on the heart muscle by the increase in vascular tone (which increases vascular resistance), but, as pointed out previously (18), may also be a maladaptive process associated with other circulating and/or cellular factors triggered by dietary sodium.

Increases in salt intake in rats are associated with increased expression of salt-inducible kinase 1 (SIK1) within the adrenal medulla (24, 49). SIK1 is a member of the AMPK family of kinases (20). Recently, we observed that SIK1 associates with the plasma membrane Na+,K+-ATPase regulatory network and thereby controls its catalytic activity upon increases in intracellular sodium (43). Na+,K+-ATPase activity is a critical cellular process required to adjust sodium homeostasis after a small, short-term elevation in intracellular sodium (43). Furthermore, blocking SIK1 activity in renal epithelial cells prevents the hypertensive cell phenotype induced by a hypertension-linked mutation in α-adducin (44). These findings show that SIK1 influences hypertension. However, several observations suggest that SIK1 may play also another role in cardiac hypertrophy. SIK1 shuttles between cytoplasm and nucleus, affecting transcription of several genes (21), in particular those associated with the synthesis of adrenal steroids and those in which transcription is mediated by CREB via the TORC-SIK1 cascade (46). In addition, lack of SIK1 in mouse embryonic stem cells impairs cardiomyogenesis by downregulating cell-cycle kinases (37). Thus this study was designed to test the hypothesis that sustained increases in intracellular sodium concentration in HL-1 cells may directly influence the activity of cardiac-specific transcription factors and hypertrophic gene expression through activation of the SIK1 network.
MATERIALS AND METHODS

Reagents. The antibody against SIK1 has been described previously (28). All Taqman probes [SIK1 Mm00440317_ml; precursor of brain natriuretic peptide (NPPB), Mm01255770_g1; α-myosin heavy chain (MHC), Mm01318344_mH; RPLP0, Mm00725448_s1] were purchased from Applied Biosystems (Foster City, CA). A23187 and monensin were obtained from Sigma. BAPTA was from Invitrogen (Carlsbad, CA). MC1658, KN-93, and KB-R7943 were from Calbiochem EMD Biosciences (San Diego, CA). All other reagents were of the highest grade available.

Plasmids. SIK1 wild-type (SIK1 WT), SIK1K56M, and SIK1T322A cDNAs are described elsewhere (43). Myocyte enhancer factor (MEF)2 and MEF2-mutant luciferase constructs were obtained from Dr. Zixu Mao (Departments of Pharmacology and Neurology, Emory University School of Medicine, Atlanta, GA) (42). NFAT luciferase (pTA-NFAT) and empty vector (pTA) were purchased from Clontech (Palo Alto, CA). The pRL-TK Renilla plasmid was obtained from Promega (Madison, WI). HDAC wild-type (HDACWT) and HDAC5 derivative (HDAC5WT) were described elsewhere (47). Brain natriuretic peptide (BNP)-tagged luciferase was obtained from David Gardner, HDACS259A are described elsewhere (47). Brain natriuretic peptide was purchased from Promega (Madison, WI). Dr. W. Claycomb) were used throughout the experiments (6). Although this mouse cell line is derived from atrial cells it expresses the cardiac myocyte phenotype and also the SIK1 isoform. These cells retain their phenotype in culture and can also be manipulated (e.g., transient transfection) without loss of the phenotype. Cells were cultured in Claycomb medium (Sigma), supplemented with 10% fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin, 1% L-glutamine, and 10 μM norepinephrine (Sigma). Cells were grown in flasks coated with fibronectin [25 μg fibronectin (Sigma) in 2 ml of 0.02% gelatin (Sigma)]. All transient transfections were performed using the lipofection method (LipofectAMINE 2000, Invitrogen) for 8 h according to the manufacturer’s instructions. Luciferase experiments were performed either by incubating with the agonists immediately after transfection or after the cells had been allowed to express the luciferase-tagged constructs for 32 h.

Determination of gene expression in biopsies from human hearts. Tissue from the intima/media layer of mammary artery (n = 88) and heart (n = 139) were collected from Swedish patients undergoing heart valve surgery (34). The local ethics committee approved the study, and informed consent was obtained from all patients. The intima/media and adventitial layers of the mammary artery were separated by adventicectomy. Biopsies were directly incubated with RNAlater (Ambion, Austin, TX) and homogenized with a FastPrep using Lysing Matrix D tubes (MP Biomedicals). Total RNA was isolated using Trizol (BRL-Life Technologies) and RNeasy Mini kit (Qiagen, Crawley, UK) including treatment with RNase-free DNase set (Qiagen) according to manufacturer’s instructions. The RNA quality was analyzed with an Agilent 2100 bioanalyzer (Agilent, Santa Clara, CA), and its quantity was measured by a NanoDrop (Thermo Scientific, Waltham, MA). The RNA samples were hybridized and scanned at the Karolinska Institutet microarray core facility. Affymetrix GeneChip Human Exon 1.0 ST arrays and protocols were used (Affymetrix, Santa Clara, CA). The raw cell files were preprocessed and log2 transformed using Robust Multichip Average normalization as implemented in the Affymetrix Power Tools 1.10.2 package apt-probeset-summarize. All investigations were done on the core meta sets of meta probes provided by Affymetrix.

Determination of MEF2/NFAT luciferase activity in isolated myocytes. Cells were replated (24-h post-transfection) with MEF2/NFAT reporters in 24-well plates. After incubations according to the desired protocols the medium was removed and cells were washed with ice-cold PBS. To obtain the cell lysates, Luciferase Assay Buffer (Promega, Madison, WI) was added to each well. The cell lysates were vortexed for 30 s and incubated at −20°C for 5 min. Cells were further disrupted on a shaker for 30 min. Cell lysates (20 μl) were mixed with Luciferase Assay Reagent. The light emitted was quantified with a Luminometer (Turner Designs, Sunnyvale, CA). For Renilla normalization Stop & Glo Reagent (Promega) was added and the light emitted by Renilla luciferase was measured. Luciferase activity was expressed as the ratio between the light emitted by MEF2-Luc or MEF2 Mut-Luc and Renilla. Luciferase activity was also expressed as percent change of the ratio between the light produced and the protein concentration in the sample (in relative light units per milligrams of protein). Protein concentration in the cell lysates was determined using a commercial dye reagent (Bradford, Bio-Rad).

In vitro phosphorylation of SIK1 peptides. Peptides for HDAC5 fragments [(S259): LRRKTAEPNLN, S498: LRRTIQSSPLP, S661: LGRRTQQSPA; S indicates the candidate for the phosphorylation site of SIK1] were expressed as glutathione S-transferase (GST)-fusion protein and used as the substrate. E. coli-expressed recombinant SIK1 (GST-SIK; 0.1 μg) was incubated with the substrates (0.1 μg) in the presence of 1.0 μCi [γ-32P]ATP at 25°C for 20 min. The reaction was stopped by the addition of sample buffer. The peptides were separated by SDS-PAGE and identified by autoradiography. GST-Syntide2 peptide was used as a positive control.

Determination of gene expression. Total cellular RNA was isolated from HL-1 cells using E.Z.N.A. Total RNA Kit I (Omega Bio-Tek, Norcross, GA). RNA was quantified spectrophotometrically using a nanodrop 1000 Spectrophotometer (Thermo Scientific). Total RNA (0.1 μg) was reverse transcribed using RevertAid H Minus M-MulV reverse transcriptase (Fermentas, Life Science, Vilnius, Lithuania). TaqMan gene expression assays and TaqMan reagents were all obtained from Applied Biosystems (Foster City, CA). Assays were performed using ABI Prism 7000 Sequence Detection System (Applied Biosystems) using five-point standard curve with a 10-fold dilution of sample PCR product according to manufacturer’s protocol. Results were expressed as percent change of units using RPLP0 mRNA expression for normalization of all assays.

Determination of intracellular pH. Cells were grown in 96-well plates, as described previously (13). The cell culture medium was aspirated, and the cell monolayers were incubated for 30 min with 10 mM BCECF/AM, the membrane-permeant acetoxymethyl ester derivative of BCECF at 37°C in 5% CO2-95% air atmosphere. Cells were placed in the sample compartment of a dual-scanning microplate spectrophotometer (Spectramax Gemini XS, Molecular Devices), and fluorescence was measured every 17 s alternating between 440 and 490 nm excitation at 535 nm emission, with a cut-off filter of 530 nm. The ratio of intracellular BCECF fluorescence at 490 and 440 nm was converted to intracellular pH (pHi) values by comparison with values from an intracellular calibration curve using the nigericin (10 μM) and high-K+ method (13, 35).

Statistical analysis. Statistical analysis was performed using GraphPad Prism (GraphPad Software, San Diego, CA). All statistical tests were performed with the unpaired Student’s t-test or ANOVA (Tukey correction) as appropriate. Differences of P < 0.05 were considered statistically significant.

RESULTS

The expression of genes that are associated with cardiomyocyte hypertrophy was examined in a cardiac atrial myocyte cell line (HL-1) upon increases in intracellular sodium...
Fig. 2. Increases in intracellular sodium increase myocyte enhancer factor (MEF2) activity. HL-1 cells were incubated with monensin (5 μM, at 37°C) or vehicle. Brain natriuretic peptide (BNP; A) and α-myosin heavy chain (α-MHC; B) mRNA expression levels were determined and expressed as percent change or fold increase. Each bar represents means ± SE of 3 to 4 determinations performed independently. BNP (C) and β-MHC (D) luciferase activity in HL-1 cells was determined in cells incubated with or without monensin (3 μM, at 37°C; BNP, 3 h and β-MHC, 12 h). Luciferase readings were corrected for protein concentration and expressed as percent increase in monensin-treated cells vs. nontreated cells. Each bar represents the mean ± SE of 4–7 experiments performed in triplicate and independently. AU, arbitrary units.

([Na+]i). This cell line expresses both BNP and α-MHC (6, 50). Increases in [Na+]i, were accomplished by using the sodium ionophore monensin, as described previously (8). The concentration used (3–5 μM) was validated in renal epithelial cells using live cell imaging, and it increased [Na+]i by ~6–10 mM under steady-state conditions (8). These changes in [Na+]i, do not affect the cell architecture or other vital functions of the cell (8, 43). In our studies, this treatment did not affect the morphology of HL-1 cells. The raising effect of monensin on intracellular sodium was validated by measuring the activity of the plasma membrane Na⁺,K⁺-ATPase. Monensin (3 μM for 15 min at room temperature) significantly increased Na⁺,K⁺-ATPase activity in HL-1 cells (nmol Pi/mg protein/min, vehicle: 21.8 ± 2.1 vs. monensin: 64.6 ± 8.1, n = 4, P = 0.0005). Monensin also induced a time-dependent increase in the levels of mRNA for BNP (Fig. 1A; P = 0.0001) and α-MHC (Fig. 1B; P = 0.01). Furthermore, luciferase-tagged reporters showed that monensin increased the expression of BNP (Fig. 1C) and β-MHC (Fig. 1D). Whereas an increase in [Na+]i, increased α-MHC, it also increased β-MHC, which is the isoform associated with increases in cardiomyocyte size. As is clear from the figure legends, different incubation times were used; the times were selected so as to obtain the maximal effect. These results clearly demonstrate that a small but sustained increase in [Na+]i, may trigger increased expression of genes associated with cardiomyocyte hypertrophy.

MEF2 has been long recognized to be a critical transcription factor that controls cardiac gene expression and is also relevant for normal cardiac growth and the development of cardiac hypertrophy (5, 7). Incubation with monensin for 3 h immediately after transfection with the MEF2-Luc reporter elicited a significant increase in MEF2-Luc activity (Fig. 2A). The effect of monensin was absent in HL-1 cells transfected with a mutant MEF2-Luc construct (lacks the MEF2 response element in the c-jun promoter). When HL-1 cells were allowed to express MEF2-Luc reporter for 32 h, incubation with monensin induced a time-dependent increase in MEF2-Luc activity (Fig. 2B), and this effect was absent in cells expressing a mutant MEF2 reporter. We also validated the effect of sodium by using low concentrations of veratridine. This agent increase MEF2-Luciferase by 63.6 ± 9.3% compared with vehicle-treated cells (Fig. 2C), and this effect was absent in cells expressing a mutant MEF2-Luc reporter.

Increases in [Na+]i, have been shown to activate, in a calcium-dependent manner, a signaling cascade that is ultimately aimed at maintaining cell sodium homeostasis (43). Among the components of this signaling network is the salt-inducible kinase 1 (SIK1). SIK1 is also essential for normal myocardial development (37, 39). Therefore, we examined the association of SIK1 mRNA expression levels with mRNA levels of genes in which expression is known to be elevated in cardiac hypertrophy. In human cardiac biopsies, the mRNA
levels of SIK1 correlated positively with those of cardiac muscle β-MHC-natriuretic peptide B and skeletal actin (Fig. 3A). Strong correlations between SIK1 expression and MYH7 (gene for β-MHC) and NPPB were also present in mammary artery (Fig. 3B). To establish whether these hypertrophic genes could be under the control of SIK1 we reduced the expression levels of SIK1 (Fig. 3C, top) in HL-1 cells using a specific SIK1 siRNA and examined the expression levels of β-MHC (Fig. 3C, middle) and skeletal actin (Fig. 3C, bottom). HL-1 cells with reduced SIK1 expression showed a significant decrease in the mRNA levels for β-MHC and skeletal actin.

It has been shown that monensin-induced increases in intracellular sodium increase protein synthesis (~35%) (22) via the Na+/Ca²⁺-exchanger (23). Reducing extracellular sodium and thereby sodium influx via the Na⁺/H⁺-exchanger also attenuates (by 35%) the increase in protein synthesis induced by calf serum (3). Because increases in [Na⁺] are associated with increases in SIK1 activity, myocardial gene expression, and MEF2 activity, we next examined whether SIK1 could have a mediating role in this process. Thus, the increases in BNP and β-MHC mRNA expression levels and MEF2 activity in response to monensin were examined in HL-1 cells transiently expressing SIK1 wild-type or the SIK1 protein in which Lys-56 has been replaced by Met-56 (K56M) (Fig. 4); this substitution renders the kinase inactive (28). The presence of an inactive SIK1 (K56M) abrogated the increases in the expression of BNP- (Fig. 4A) and β-MHC-luciferase (Fig. 4B) induced by increases in [Na⁺]. Similarly, basal MEF2 activity was significantly decreased in cells overexpressing the SIK1-K56M mutant or SIK1-T322A (lacking the CaMK1 phosphorylation/activation site) (43) compared with cells overexpressing the SIK1 wild-type (Fig. 4C), suggesting that basal MEF2 activity is under the control of SIK1. Furthermore, incubation with monensin did not result in increases in MEF2 activity (Fig. 4D). These results illustrate the potential role of SIK1 in HL-1 cells during the regulation of MEF2 activity and BNP/β-MHC expression in response to increases in [Na⁺].
Because monensin had no effect on MEF2 activity in the presence of the SIK1-T322A mutant (which lacks the CaMK1 phosphorylation domain), we explored the calcium dependency of the network activation and its response in HL-1 cells (Fig. 5). Increases in \( [\text{Na}^+]_i \) are paralleled by increases in intracellular calcium in both renal epithelial cells (8) and cardiac myocytes (55). Therefore, to examine the calcium dependency of MEF2 activation, HL-1 cells were incubated with BAPTA/EGTA, which prevents the increases in intracellular calcium elicited by monensin (8). Under these conditions, the effect of monensin on MEF2 activity was significantly reduced (Fig. 5A). Similarly, blocking the \( \text{Na}^+/\text{Ca}^{2+} \) exchanger (Fig. 5B) or the calcium calmodulin kinase (Fig. 5C) significantly reduced the effect of monensin on MEF2 activity. CaMKII can also directly activate MEF2. Increasing intracellular calcium with a calcium ionophore does not result in increases in SIK1 activity (43), but it does increase MEF2 activity (Fig. 5D), suggesting that MEF2 can be activated via two different pathways (SIK1-dependent and -independent) and that the SIK1-dependent pathway is responsible for the response to increases in intracellular sodium. The latter could be the result of SIK1 interacting with other (yet unknown) signaling mediators, and/or due to a compartmentalization of the signal. In HL-1 cells, increases in \( [\text{Na}^+]_i \), induced by monensin are associated with transient (5–20 min) intracellular alkalization (Table 1), which is absent at 6 h incubation (when gene expression was measured). To examine whether the changes in \( \text{pH}_i \) could be partially responsible for the effect of intracellular sodium on MEF2 luciferase activity, HL-1 cells were incubated with monensin in the presence or absence \( \text{NaHCO}_3 \). At 25 mM, \( \text{NaHCO}_3 \) increased \( \text{pH}_i \) by \(-0.3 \) units. In the absence of \( \text{NaHCO}_3 \) monensin increased the \( \text{pH}_i \) by 0.196 units, whereas in the presence of \( \text{NaHCO}_3 \) the increase was only 0.007 units (Fig. 5E). Under these conditions, there was no significant difference in basal or monensin-stimulated increases in MEF2 luciferase activity (Fig. 5F), suggesting that changes in \( \text{pH}_i \), induced by \( [\text{Na}^+]_i \), per se do influence the regulation of transcriptional activity.

The mechanisms by which SIK1 controls MEF2 activity were further examined. HDACs are key controllers of heart...
muscle development (57). In skeletal muscle, SIK1 promotes MEF2 activation by phosphorylating HDAC Class II and dissociating it from MEF2 (2). First, we examined the effect of monensin on intracellular pH, HL-1 cells were transiently transformed with a wild-type (Fig. 7B), suggesting a potential MEF2 dependency in the activation of NFAT.  

DISCUSSION  

These studies performed in a cell model derived from mouse cardiac atrial myocytes demonstrate that increases in sodium permeability can trigger, in a calcium-dependent manner, the activation of transcription factors and genes that are associated both with normal cardiac growth and cardiomyocyte hypertrophy. Furthermore, a sodium-sensing network with the SIK1 at its core mediates the cellular effects of sodium.  

Although numerous studies performed in plants highlight the influence of intracellular sodium in triggering the expression of numerous genes, very little information has been obtained in animal cells, except for studies in which sodium has been shown to affect the expression of subunits of Na⁺,K⁺-ATPase, a crucial enzyme responsible for cell sodium homeostasis (54). In cardiac myocytes, indirect evidence was obtained by using ouabain and digitalis at concentrations that increase [Na⁺], (by inhibiting the Na⁺,K⁺-ATPase activity) (53). Both agents promote hypertrophic effects and increases in the expression of the genes encoding BNP and the Na⁺/H⁺-exchanger. Moreover, these effects were attenuated upon inhibition of the Na⁺/H⁺-exchanger (11), suggesting that either changes in [Na⁺], or pH were possible mediators. A previous study has also convincingly demonstrated that stretch-dependent increases in [Na⁺] could represent an early signal leading to abnormal myocardial growth (22). The results from our studies clearly support the notion that small increases in intracellular sodium affect the expression of hypertrophic genes (BNP/MHC) and transcription factors (MEF2/NFAT). Furthermore, they provide part of the molecular mechanism (Fig. 8): increases in [Na⁺], via the reverse Na⁺/Ca²⁺-exchanger present...

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**Table 1. Effect of monensin on intracellular pH**

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<th>Time and Condition</th>
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<td>5 Min</td>
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<td></td>
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<tr>
<td>Vehicle</td>
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<tr>
<td>Monensin</td>
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<tr>
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<td>17</td>
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<tr>
<td>Vehicle</td>
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<tr>
<td>Monensin</td>
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<td>20 min</td>
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<td>Monensin</td>
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**Fig. 6.** Mechanisms by which SIK1 controls MEF2 activity in response to elevated intracellular sodium. A: effect of monensin (Mon; 5 μM, for 15 min at 23°C) on MEF2 activity was examined in HL-1 cells treated previously with MC1568 (1 μM, for 24 h at 23°C) or vehicle. Each bar represents means ± SE of 6 experiments performed in triplicates. B: SIK1-dependent phosphorylation of HDAC5 peptides (S259: LRKTA, S498: LSRTQ, S661: LGRTQSPAA; S indicates the candidate phosphorylation site of SIK1). C: effect of monensin (5 μM, for 15 min at 23°C) on MEF2 activity was examined in HL-1 cells transiently expressing HDAC5 WT or the HDAC5 mutant (S259A). MEF2 activity is expressed as percent change (monensin vs. vehicle). Each bar represents means ± SE of 6–7 experiments performed independently and in triplicates.
changes in intracellular Ca\textsuperscript{2+} have profound effects on transcription, gene expression, and myocardial growth (4). The actions of calcium within myocytes are translated via the activation of distinct signaling networks involving Ca\textsuperscript{2+}-dependent kinases (29) and phosphatases (calcineurin) (51). However, the majority of these pathways are not clearly proven to contribute significantly to abnormal cardiac growth in response to high salt intake or salt-sensitive hypertension. In this study, we provide evidence for an additional/different level of interaction that includes the activation of SIK1 via CaMK1 phosphorylation (Fig. 8). Although this network is activated by increased sodium permeability, it is also plausible that it could be activated by hormones (such as angiotensin II, aldosterone), thereby influencing abnormal myocardial growth.

Increases in [Na\textsuperscript{+}], activate MEF2/NFAT and increase the expression of MHC/BNP genes. The possibility that MEF2 or NFAT directly controls the expression levels of MHC/BNP upon elevations in intracellular sodium was not explored in this study. However, it has been reported previously that cardiac α-MHC and atrial natriuretic factor genes are under the control of MEF2 directly or in association with other transcription factors such as GATA-4 (27, 31, 38). In addition, the BNP promoter is known to be a target for NFATc3, and similarly to MEF2, NFATc3 could also act alone or in synergy with GATA-4 (14). The fact that these transcription factors involved in heart development can be activated by mutual synergisms and have mutual potential interacting domains is interesting and may resolve the issue of redundancy, where signals may affect the activity of multiple transcription factors cascades by regulating just one of them. Besides being involved in the development of cardiac hypertrophy, NFAT5 is also known as tonicity-responsive enhancer binding protein. Because it is activated during the cellular response to osmotic stress in the renal epithelia (38) as well as part of some immune responses (25), it is not surprising that its activity may also be controlled by rises in [Na\textsuperscript{+}].

The mechanisms by which SIK1 controls MEF2 activity involved the phosphorylation of HDAC. This phenomenon has also been reported previously in Caenorhabditis elegans (48) and human skeletal muscle (14). Sodium-dependent regulation of HDAC could have an impact that extends beyond the activation of transcription. Apart from MEF2 activation, SIK1-HDAC5 could affect chromatin remodeling and, by enhanced methylation of other relevant targets, affect cell growth and division (56). Nevertheless, the molecular mechanisms beyond

in HL-1 cells. Other means of calcium entry across the plasma membrane, such as voltage-dependent \( L \)-type C channels, TRPC cation channels or calcium release from intracellular stores via InsP\textsubscript{3} receptors-Ca\textsuperscript{2+} channels, ryanodine receptors (12, 19, 52) have been demonstrated to affect transcription and myocardial growth in response to hormone signals or pressure overload. Whether these signals affect transcription via SIK1 remains to be explored. Multiple pathways of calcium entry
HDAC phosphorylation in response to elevated intracellular sodium are complex and deserve further and extensive evaluation. The cellular mechanism involved in the activation of NFAT by the SIK1 network is less clear and deserves further investigation. It is possible that NFAT is directly regulated by SIK1-dependent phosphorylation, because the NFAT protein bears a potential SIK1 consensus phosphorylation domain (I-F-R-T-S-S\(^{417}\)-L-P-P-L). Alternatively, and because the activation of NFAT is blocked by the HDAC mutant (lacking the SIK1 phosphorylation motif), such an increase in activity could be due to a synergistic effect of MEF2 as suggested previously in other cell models (7, 36) or could be exerted via an independent HDAC/NFAT pathway. The activation of hypertrophic genes by sodium may require the combined activation of multiple transcription factors.

Increased dietary salt intake is associated with increases in SIK1 expression in animal models (49). In this study, increases in intracellular sodium increased SIK1 expression. We have not examined the potential cellular events leading to increase in SIK1 expression; however, the data may suggest that either salt, or other humoral factors associated with high salt intake, may be responsible for this effect. It can be speculated that events associated with chronic elevation in arterial blood pressure—and not just the pressure effect per se—may influence cardiac growth. Essential hypertension is associated with sodium retention, plasma volume expansion, release of ouabain-like factors, and vasoconstriction. In this condition, the concentration of sodium in blood (natremia) is normal, but the absolute amount of sodium in the body is higher. A higher absolute amount of sodium may affect directly or indirectly the intracellular sodium concentration, with deleterious effects.

In conclusion, our results support the role of increased intracellular sodium concentration as an independent regulator of transcription and activation of gene expression in a cell line derived from atrial myocytes. Furthermore, SIK1 appears to be an essential mediator of these effects.

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