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Antagonistic modulation of SIK1 and SIK2 isoforms in high blood pressure and cardiac hypertrophy triggered by high-salt intake

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ABSTRACT

Salt-inducible kinases (SIKs) represent a subfamily of AMPK family kinases. SIK1 has been shown to act as a mediator during the cellular adaptation to variations in intracellular sodium in a variety of cell types. SIK2, as an isoform of the SIK family, modulates various biological functions and acts as a signal transmitter in various pathways. To evaluate the role of both SIK1 and SIK2 isoforms in blood pressure (BP), body fluid regulation and cardiac hypertrophy development, we made use of constitutive *sik1*$^{-/-}$ (SIK1-KO), *sik2*$^{-/-}$ (SIK2-KO), double *sik1*$^{-/-}$*sik2*$^{-/-}$ (double SIK1*2-KO) knockout and wild-type (WT) mice challenged to a standard (0.3% NaCl) or chronic high-salt (HS, 8% NaCl) diet intake for 12 weeks.

Mice, under a standard diet intake, had similar and normal BP. On a chronic HS intake, SIK1-KO and double SIK1*2-KO mice showed increased BP, but not WT and SIK2-KO mice. A chronic HS intake led to the development of cardiac left ventricle hypertrophy (LVH) in normotensive WT and hypertensive SIK1-KO mice, but not in SIK2-KO mice. Double SIK1*2-KO mice under standard diet intake show normal BP but an increased LV mass. Remarkably, in response to a dietary stress condition, there is an increase in BP but LVH remained unchanged in double SIK1*2-KO mice.

In summary, SIK1 isoform is required for maintaining normal BP in response to HS intake. LVH triggered by HS intake requires SIK2 isoform and is independent of high BP.

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Introduction

Salt-inducible kinases (SIKs) represent a subfamily of AMP-activated protein kinase (AMPK) family kinases (1). Within the AMPK family, the subfamily of SIKs contains three kinases (SIK1, SIK2, and SIK3). SIK1, the founding member of this subfamily, was identified and named as a kinase whose expression is induced in the adrenal gland of rats fed a high-salt (HS) diet (2). Subsequent homology searches led to the identification of SIK2 and SIK3 and all three SIK family kinases are expressed broadly (3–5). As an isoform of the SIK family, SIK2 modulates various biological functions and acts as a signal transmitter in various pathways (3,6). Compared with that in adjacent normal tissues, the expression of SIK2 is significantly higher in multiple types of tumors, which indicates its pivotal effect in oncogenesis (7).

Hypertension is the result of complex interactions between genetic and environmental factors. Despite this, it is thought that renal mechanisms play a primary role in blood pressure (BP) increase through impairment of sodium and water handling. SIK1 was initially discovered in adrenal glands of rats fed with an HS diet and it has been shown to act as a mediator during the cellular adaptation to variations in intracellular sodium in a variety of cell types (8). SIK1 participates in sodium reabsorption in the proximal convoluted tubule via modulating Na^+,K^+-ATPase (NKA) activity and is involved in the establishment of a polarized epithelium, which is essential for the vectorial transport of ions and solutes via controlling E-cadherin availability in the plasma membrane (9). Loss of SIK1 contributes to the development of high BP, vascular remodeling and cardiac hypertrophy triggered by a chronic (14-week) HS intake in mice. The negative impact on BP is associated with alterations of the vasculature and the regulatory action of SIK1 on BP levels are exerted specifically during abnormal salt intake (10).

Lack of SIK2 in response to chronic (12-week) HS intake is not accompanied by increases in BP, but prevents the development of cardiac hypertrophy in mice (11). SIK2 is responsible for the cellular/molecular events in cardiac myocytes that lead to cardiac hypertrophy. Blocking SIK2 activity may be an important way to prevent the increase in left ventricle mass (LVM) occurring in response to a HS intake and/or to abnormal elevations in arterial BP indicating that SIK2 isoform, rather than SIK1, is more relevant for abnormal LV growth (11).

More recently, the increase in systolic BP in *sik1*$^{-/-}$ (SIK1-KO) mice after 7-day HS intake was reported to be accompanied by overactivity of the sympathetic nervous system, no changes in serum renin and angiotensin II levels, but decreases in aldosterone serum levels (12). This phenotype in SIK1-KO mice on HS intake is accompanied by marked natriuresis with normal plasma Na^+ and K^+ levels (10,12). In *sik2*$^{-/-}$ (SIK2-KO) mice on HS intake

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normal plasma Na^+ and K^+ levels have also been reported (11).

The apparent antagonistic role of the two SIK isoforms (SIK1 and SIK2) leads to question whether the absence of SIK2 could prevent the deleterious effect caused by the absence of SIK1 on the cardiovascular system, namely, the development of cardiac hypertrophy (ie, LVM) in response to chronic HS intake. For this purpose, we generated a novel double *sik1*$^{-/-}$*sik2*$^{-/-}$ (SIK1*2-KO) mouse model. Here, we evaluated tissue remodeling and cardiac-related parameters and the renal function in SIK1-KO, SIK2-KO, SIK1*2-KO and WT mice challenged to a normal salt (0.3% NaCl) or chronic HS (8% NaCl) diet intake for 12 weeks.

Materials and methods

Animal care and general procedures

Constitutive SIK1-KO mice were generated by Taconic Biosciences, Inc (Hudson, USA) and previously described (9). Constitutive SIK2-KO mice were generated by PolyGene AG (Rümlang, Switzerland) by targeted mutation using a C57BL/6 embryonic stem cell line. Offspring was maintained on a C57Bl/6 background. Female SIK1-KO mice were bred with male SIK2-KO mice. The resulting double heterozygous offspring were further crossed to establish four homozygous lines: wild-type (WT), SIK1-KO, SIK2-KO and SIK1*2-KO mice. Offspring was inbred to at least four generations. Animals do not show any phenotype difference or any difference in their reproduction or life span. Colony was maintained on a homozygous breeding scheme.

Animals were housed in macrolon cages (Tecniplast, Varese, Italy) with free access to food (#2014 Teklad Global Rodent Diets®, Envigo, Barcelona, Spain) and tap water under controlled environmental conditions in a colony room (12 h light/dark cycle, room temperature: 22 ± 2°C and relative humidity: 50 ± 20%) until the beginning of the experiments. Eight-week-old male mice were challenged either a HS (8% NaCl, #D02011103) or control (0.3% NaCl, #D02112603) AIN-76A rodent diet (Research Diets Inc., New Brunswick, NJ, USA) for 12 weeks. Animal procedures conformed to the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes and the Portuguese law on animal welfare (Decreto-Lei 113/2013 and 1/2019).

Blood pressure measurements

Mice were instrumented with radio-telemeters (TA11PA-C10, Data Sciences International, St Paul, MN, USA), for BP, heart rate, and home-cage activity assessment at the end of the 12-week diet run-in period, as described elsewhere (12). Briefly, telemetry transmitter was inserted into the carotid artery after cranial permanent ligature and temporary caudal occlusion. Catheter tip was positioned and secured in the aortic arch. Animals recovered individually for 3–5 days before recordings. After recovery, telemetry probes were magnetically turned-on and BP, heart rate and homecage locomotor activity were monitored. Raw data were recorded for 40 s every 10 min for a 24-h period using Dataquest A. R.T. Acquisition and Analysis system 4.0 (Data Sciences International).

Renal function

Mice were individually placed in mouse metabolic cages (Tecniplast) for a 24-h urine collection. The volume of water intake and urinary excretion was noted. The urine samples were collected and stored at −80°C until assayed. Urinary creatinine levels were determined using an enzymatic colorimetric test (HUMAN Diagnostics, Wiesbaden, Germany; #10051). The level of proteins in urine was determined by a photometric assay (Bio-Rad, Hercules, CA, USA). Urinary electrolytes determination was performed by Cobas Mira Plus analyzer (ABX Diagnostics for Cobas Mira, Switzerland). All analyses were run in duplicate acceding to manufacturer´s instructions.

Remodeling evaluation

At the end of the study, mice were sacrificed with an overdose of pentobarbital sodium. Heart (ventricles + septum), kidneys, lungs, liver and spleen were collected, blotted dry and weighed. The heart-, left ventricle-, right ventricle-, kidney-, lung-, liver- and spleen-to-body weight ratios were calculated and used as an index of hypertrophy.

Plasma markers

Blood samples were withdrawn from each animal at the end of study. Plasma creatinine and urea levels were determined using enzymatic colorimetric tests (HUMAN Diagnostics, Wiesbaden, Germany; #10051 and #10505, respectively). Plasma levels of glucose, triglycerides and total cholesterol were evaluated using enzymatic colorimetric tests (HUMAN Diagnostics, Wiesbaden, Germany, #10260, #10725 and #10028, respectively). Plasma electrolytes determination was performed by Cobas Mira Plus analyzer. All analyses were run in duplicate acceding to manufacturer´s instructions.

Data analysis and statistical methods

The data are presented as the mean ± SEM. Data analyses were performed using Prism 8 (GraphPad Software, San Diego, CA, USA). The data were analyzed by two-way analysis of variance (ANOVA) followed by multiple comparison test, as appropriate. A value of $P < .05$ was considered statistically significant.

Results

Water intake and urinalysis in WT, SIK1-KO, SIK2-KO and SIK1*2-KO mice on a control and HS diet intake for 12 weeks are summarized in Table 1. Water intake and urinary volume output were similar between genotypes under control salt intake. Upon HS intake, the water consumption increased by 3.4-, 2.5-, 2.3- and 2.2-fold in WT, SIK1-KO, SIK2-KO and SIK1*2-KO mice, respectively, and this was accompanied by an increase in urinary volume output. Under control diet feeding and HS intake, creatinine clearance and plasma urea was not

Table 1. Renal function evaluation after 12 weeks of 0.3% or 8% NaCl intake.

	0.3% NaCl diet				8% NaCl diet			
	WT	SIK1-KO	SIK2-KO	SIK1*2-KO	WT	SIK1-KO	SIK2-KO	SIK1*2-KO
Water intake (ml/24h)	3.2±0.6	3.8±0.9	2.7±0.6	3.8±0.5	11.1±1.6 $^{\#}$	9.4±0.7 $^{\#}$	6.3±0.6 $^{*\ \#}$	8.3±1.1 $^{\#}$
Urine output (ml/24h)	0.8±0.1	1.3±0.2	1.5±0.2	1.9±0.4	6.3±1.2 $^{\#}$	6.3±0.6 $^{\#}$	3.6±0.8 $^{*\ ‡}$	4.8±0.4 $^{\#}$
Plasma urea (mg/dl)	41.9±3.8	34.6±1.3	37.9±2.8	41.0±0.8	43.6±3.0	40.7±1.3	44.4±2.0	54.0±6.2 $^{\#}$
Creatinene Clearance (µl/min)	46.2±8.3	61.2±16.5	68.5±4.5	74.8±9.5	63.5±6.4	64.0±8.5	56.7±7.9	52.0±8.9
Urine protein (mg/24h)	1.97±0.32	2.50±0.49	2.13±0.15	2.73±0.40	3.33±0.47 $^{\#}$	3.42±0.32	2.04±0.29	2.77±0.35
FE_{Na} (%)	0.46±0.03	0.69±0.12	0.55±0.05	0.89±0.29	24.6±3.1 $^{\#}$	29.3±1.7 $^{\#}$	18.4±2.2 $^{\#}$	34.6±6.0 $^{\#}$
FE_{K} (%)	23.9±2.2	38.0±4.9	28.0±1.3	44.3±5.9	51.9±4.1 $^{\#}$	62.4±11.2	43.8±4.4	77.3±14.7 $^{*\ †\ \#}$
Fe_{Cl} (%)	1.83±0.23	2.79±0.36	2.00±0.10	2.18±0.22	30.1±5.6 $^{\#}$	44.9±14.1 $^{\#}$	31.9±7.6 $^{\#}$	26.9±5.0 $^{\#}$

Values are Mean ± SEM of N = 3 to 8/group.
$^{*}P < .05$ vs. WT mice (2-way ANOVA followed by Tukey´s multiple comparisons test).
$^{‡}P < .05$ vs. SIK1-KO mice (2-way ANOVA followed by Tukey´s multiple comparisons test).
$^{†}P < .05$ vs. SIK2-KO mice (2-way ANOVA followed by Tukey´s multiple comparisons test).
$^{\#}P < .05$ vs. 0.3% NaCl diet-fed counterparts (2-way ANOVA followed by Sidak´s multiple comparisons test).

different between WT, SIK1-KO, SIK2-KO and SIK1*2-KO mice. SIK1 or SIK2 ablation had no effect on urinary protein, but SIK1-KO and SIK1*2-KO mice excreted less protein in the urine than WT mice on HS intake, but not on control diet. Increases in plasma urea were observed in SIK1*2-KO mice on a HS intake. No differences between genotypes were observed in the fractional excretion of sodium, potassium or chloride on a control diet. A HS intake significantly increased the fractional excretion sodium and chloride output in all genotypes. Conversely, the fractional excretion of potassium in SIK2-KO were lower than in WT mice after a HS intake.

Levels of glucose, total cholesterol and triglycerides were similar between genotypes under control salt intake. Upon HS intake, plasma glucose increased in WT and SIK1-KO, but not in SIK2-KO and SIK1*2-KO mice, respectively. Minor changes in total cholesterol were observed in SIK2-KO mice when challenged with the HS intake (Table 2).

Blood pressure and heart rate recordings were performed on telemetered in WT, SIK1-KO, SIK2-KO, and SIK1*2-KO mice on a control and HS diet intake for 12 weeks. No differences in BP were observed between in WT, SIK1-KO, SIK2-KO, and SIK1*2-KO mice on a control diet (Table 3). However, after 12 weeks of HS intake, systolic blood pressure was significantly elevated in SIK1-KO (145 ± 2 mmHg) and SIK1*2-KO mice (146 ± 4 mmHg) as compared to WT mice (128 ± 4 mmHg, $P = .05$) and to SIK2-KO mice on a control diet (131 ± 4 mmHg, $P = .05$). Diastolic blood pressure was similar in all groups after 12 weeks on HS intake (Table 3). No differences in heart rate and home-cage activity were observed between groups (Table 3).

Cardiac, kidney, lung, liver and spleen morphometric evaluations were also performed in WT, SIK1-KO, SIK2-KO, and SIK1*2-KO mice on a control and HS intake for 12 weeks (Figure 1). Body weight in SIK2-KO mice fed a control diet were slightly but significantly heavier than SIK1-KO and SIK1*2-KO mice and after 12 weeks HS intake there was a general tendency for a decrease in body weight across all genotypes, this resulting in a statistically significant difference in SIK2-KO and SIK1*2-KO mice (Figure 1A). Heart- and left ventricle-to-body weight ratios in SIK1-KO mice were significantly higher under HS intake as well as in SIK1*2-KO mice on an HS intake when compared to SIK2-KO mice (Figure 1B and 1C), whereas no changes were observed between SIK1-KO and SIK2-KO mice in right ventricle-to-body weight ratio (Figure 1D). Kidney-to-body weight ratio in SIK1-KO mice was significantly higher under HS intake as well as in SIK1*2-KO mice on an HS intake when compared to SIK2-KO mice (Figure 1E). Lung-to-body weight ratio in SIK1-KO and SIK1*2-KO mice was significantly higher as compared to SIK2-KO mice in both conditions of salt-intake (Figure 1F). No marked changes in liver- and spleen-to-body weight ratios (Figure 1G, 1H) were observed under both diet regimens for 12 weeks.

Discussion

Here, we proposed to study the role of SIK1 and SIK2 on BP rise, tissue remodeling and cardiac-related parameters, and the renal function after a long-term HS intake. The results reported confirm and extend our previous observations that vascular SIK1 activation might represent a mechanism involved in the prevention of high BP (10,12,13) and the observed increase in systolic BP in SIK1-KO and SIK1*2-KO mice under HS intake might reflect changes in arterial stiffness. Moreover, cardiac hypertrophy triggered by HS requires the SIK2 isoform and is independent of high BP.

NKA activity is modulated by SIK1 and contributes to the reabsorption of sodium in the kidney proximal tubules (9,-13–15). This is in line with the increased natriuresis observed after 7 days on HS intake, where SIK1-KO mice excreted 1.5-fold more sodium than WT mice on the same diet regimen, as creatinine excretion rates were similar (10). This difference in renal electrolyte handling should have led to a lower BP in SIK1-KO mice as compared to WT mice feed an HS diet. Nevertheless, the contribution of SIK1 in controlling the vascular tone seems to surpass the contribution of renal SIK1 which could also be compensated by other SIK isoforms mechanisms, leading to a higher BP in SIK1-KO mice than in WT mice under a HS diet (10). In an attempt to uncover the mechanisms that may be responsible for the increase in BP upon HS intake in the SIK1-KO mice, increases in the activity of renin-angiotensin-aldosterone system was discarded as a potential trigger for the rise in BP, but an overdrive of the sympathetic nervous system, namely the noradrenergic and adrenergic tone in the SIK1-KO mice may partially explain the increase in BP triggered by an HS intake which is also consistent with the overactivation of the enzyme dopamine β-hydroxylase (12).

The present study shows that heart and left ventricle mass in SIK1-KO and SIK1*2-KO mice under normal salt intake was higher than in SIK2-KO and WT mice and HS tended to increase such differences, this being particularly evident with regards to the left ventricular mass. Interestingly, such differences both during normal salt and HS intakes were not observed in the right ventricle. Experiments using *sik2*$^{-/-}$ and *sik2*$^{+/+}$ mice showed a direct link between the presence of SIK2 and the development of cardiac hypertrophy triggered by chronic HS intake (11). This study suggested that blocking SIK2 activity/expression was an important way to prevent the increase in left ventricular mass occurring in response to a HS intake and/or to abnormal elevations in arterial BP (11). Though this study demonstrated the importance of SIK2 in cardiac myocytes, it did not exclude the possibility that SIK2 present in other cell types could also be of relevance by affecting the release and/or the action of unknown cellular mediators activated in response to the higher content of salt in the diet. The present work confirmed the relevance of SIK2 for the development of left ventricular mass in the context of high salt intake and its independence from increases in BP. On the other hand, the present work extended such concept, by demonstrating that this may also apply to the kidney and lung, as shown by differences in kidney- and lung-to-body weight ratios between SIK1-KO and SIK2-KO mice. It should be underscored that in the case of the kidney and lung such differences could be observed both in mice fed a normal salt diet and during HS intake. Also of interest, are the observations that such relevance of SIK2 in cell hypertrophy, first, was not

Table 2. Glucose and lipids evaluation after 12 weeks of 0.3% or 8% NaCl intake.

	0.3% NaCl diet				8% NaCl diet			
	WT	SIK1-KO	SIK2-KO	SIK1*2-KO	WT	SIK1-KO	SIK2-KO	SIK1*2-KO
Plasma glucose (mg/dL)	202.3±5.8	208.9±16.7	215.3±6.0	236.9±7.0	318.3±13.3 $^{\#}$	388.8±28.3 $^{\#}$	269.2±37.5 ‡	240.9±21.9 *‡
Total plasma cholesterol (mg/dL)	71.6±2.5	80.5±3.5	87.7±4.2	84.0±3.8	80.6±3.9	97.0±3.7	68.3±9.2 $^{\ddagger\#}$	91.5±0.5 †
Plasma triglicerydes (mg/dL)	47.5±2.3	40.9±2.7	57.0±7.1	43.6±4.0	42.1±2.8	56.2±4.6	48.8±4.4	42.0±3.1

Values are Mean ± SEM of N = 6 to 11/group.
$^{*}P < .05$ vs. WT mice (2-way ANOVA followed by Tukey´s multiple comparisons test).
$^{\dagger}P < .05$ vs. SIK2-KO mice (2-way ANOVA followed by Tukey´s multiple comparisons test).
$^{\#}P < .05$ vs. 0.3% NaCl diet-fed counterparts (2-way ANOVA followed by Sidak´s multiple comparisons test).

Table 3. Blood pressure evaluation after 12 weeks of 0.3% or 8% NaCl intake.

	0.3% NaCl diet				8% NaCl diet			
	WT	SIK1-KO	SIK2-KO	double SIK1*2-KO	WT	SIK1-KO	SIK2-KO	double SIK1*2-KO
SBP (mm Hg)	123.6±2.2	138.6±4.5	126.3±4.0	134.0±5.9	127.9±4.1	145±1.7 *	130.6±4.3	146.2±4.4 * †
DBP (mm Hg)	96.8±3.2	101.5±4.8	93.3±2.0	97.0±2.4	99.5±3.9	108.7±2.6	96.5±3.2	106.7±3.8
MAP (mm Hg)	110.1±2.2	119.4±4.2	111.0±3.7	115.4±4.0	113.5±3.8	125.8±2.0	113.2±3.5	124.2±3.1
HR (beats/min)	562.9±18.9	568.7±10.8	566.6±10.0	554.9±27.6	545.1±9.6	525.2±17.3	552.8±10.6	498.2±11.5 †
Activity (arbitrary unit)	5.6±1.1	3.2±0.8	2.4±0.4 *	2.9±1.0	4.6±0.8	4.1±1.3	3.0±0.8	2.7±0.7

Values are Mean±SEM of N = 5 to 10/group.
SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean blood pressure; HR = heart rate.
*$P < .05$ vs. WT mice (2-way ANOVA followed by Tukey´s multiple comparisons test).
†$P < .05$ vs. SIK2-KO mice (2-way ANOVA followed by Tukey´s multiple comparisons test).
#$P < .05$ vs. 0.3% NaCl diet-fed counterparts (2-way ANOVA followed by Sidak´s multiple comparisons test).

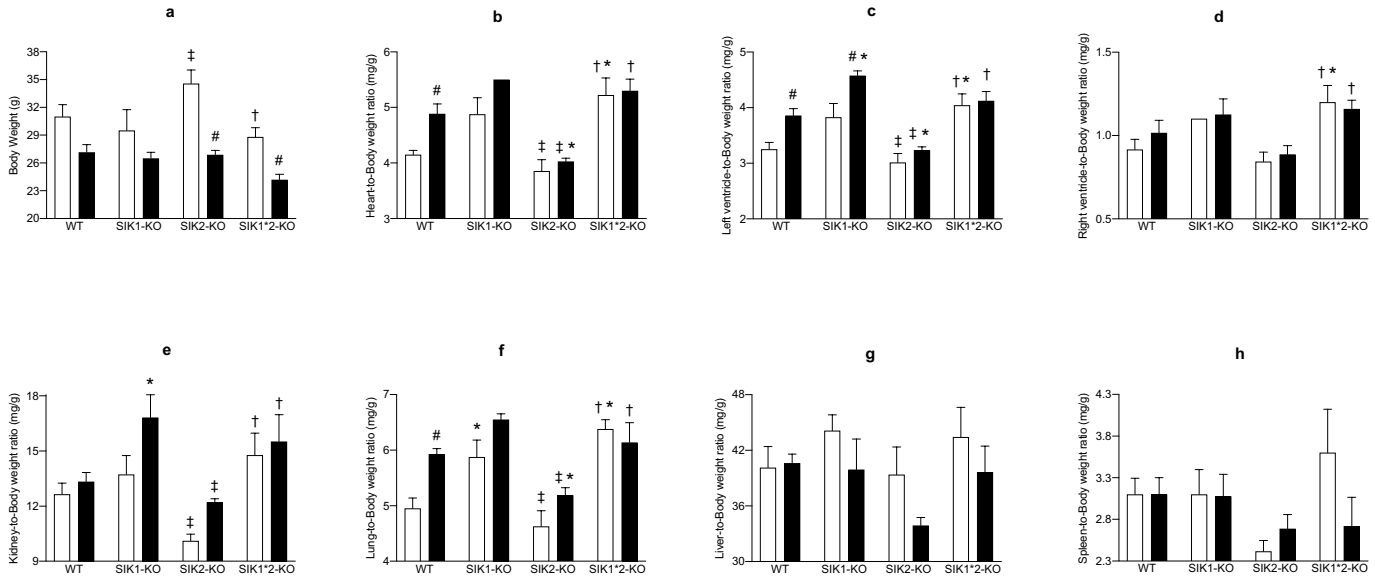

Figure 1. (**A**) Body weight and relative weights of (**B**) heart, (**C**) left ventricle, (**D**) right ventricle, (**E**) kidney, (**F**) lung, (**G**) liver and (**H**) spleen of wild-type (WT), *sik1*$^{-/-}$ (SIK1-KO), *sik2*$^{-/-}$ (SIK2-KO) and *sik1*$^{-/-}$*sik2*$^{-/-}$ (SIK1*2-KO) mice fed a normal (0.3% NaCl, open bars) or high-salt (8% NaCl, close bars) diet for 12 weeks. Columns represent means and vertical lines show SEM (n = 3–8 per group). *$P < .05$ vs. WT mice (2-way ANOVA followed by Tukey´s multiple comparisons test). ‡$P < .05$ vs. SIK1-KO mice (2-way ANOVA followed by Tukey´s multiple comparisons test). †$P < .05$ vs. SIK2-KO mice (2-way ANOVA followed by Tukey´s multiple comparisons test). #$P < .05$ vs. 0.3% NaCl diet-fed counterparts (2-way ANOVA followed by Sidak´s multiple comparisons test).

observed in the liver, spleen and right ventricle and, secondly, was not evident when both SIK1 and SIK2 genes were ablated, namely in the left ventricle, kidney and lung.

At the molecular level, one of the markers of cardiac hypertrophy in the left ventricle from *sik2*$^{+/+}$ versus *sik2*$^{-/-}$ mice on HS intake was suggested to be associated with the high expression of beta-myosin heavy chain (beta-MHC) (11). The relative contribution of SIK2 should be studied in kidney and lung cells where cell hypertrophy eventually does not associate with increases in the beta-MHC, as this contractile protein does not play a major role in kidney and lung epithelia. However, even without this information, it appears that blocking SIK2 activity/expression may be an important way to prevent the increase in left ventricular mass occurring in response to a HS intake and/or to abnormal elevations in BP. Another point that is worthwhile to mention concerns the unlikely role of volume expansion to explain increases in kidney- and lung-to-body weight ratios, as body weight was slightly lower in mice on HS intake than in those fed a normal salt diet.

In summary, SIK1 isoform is required for maintaining normal BP in response to HS intake. LVH triggered by HS intake requires SIK2 isoform and is independent of high BP.

Authorship contributions

NMP, BI and PSS participated in research design, performed data analysis and wrote or contributed to the writing of the manuscript. NMP and BI conducted experiments. All authors have contributed to the discussion of the results and have approved the final manuscript.

Declaration of competing interest

NMP, BI and PSS were employees of BIAL at the time of the studies.

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