From Membrane Signaling Networks,
Atherosclerosis Research Unit,
Department of Medicine,
Karolinska Institute, Stockholm, Sweden

Relevance of the salt-inducible kinase network for the development of high blood pressure and cardiac hypertrophy

Sergej Popov

Karolinska Institutet

Stockholm 2012

All previously published articles were reproduced with permission from the publisher.
Figures 1, 2 and 3 were produced using Servier Medical Art.
Published by Karolinska Institutet. Printed by US-AB.

ISBN 978-91-7457-827-0

ABSTRACT

Hypertension (high blood pressure) is a multifactorial condition that remains a big worldwide problem. 95% of all hypertensive people suffer from essential hypertension with unknown causes. This can cause different heart diseases such as cardiac hypertrophy (CH), a condition which can ultimately lead to heart failure. Salt intake has been suggested to be one of the factors promoting hypertension. The aims of this thesis were to elucidate the importance of salt-inducible kinase 1 (SIK1) in the development of high blood pressure (BP) and to identify salt-activated molecular pathways that could activate transcription factors and genes coupled with development of CH independently of high BP.

SIK1 has been implicated in intracellular pathways that control cell sodium homeostasis and is also important for myocyte development. To investigate whether SIK1 is a potential regulator of BP, genotype-phenotype association studies between a genetic variation within SIK1 coding region (rs3746951) and BP in four population-based cohorts were performed. After performing a meta-analysis of the four studies the results revealed an association between the rs3746951 and lower systolic- and diastolic blood pressures. Rs3746951 was also associated with lower left ventricular mass. In studies performed in cultured vascular smooth muscle cells it was shown that rs3746951 was associated with higher SIK1 and Na^+/K^+-ATPase activity. These results suggest that SIK1 affects BP.

To explore the involvement of SIK1 in the development of CH, studies were performed in a mouse cardiac atrium cell line (HL-1). The overall results revealed that SIK1 mediated the effect of salt on transcription factors MEF2 and NFAT and on the genes associated with CH: *Mhc Bnp* and *Ska*. Studies performed on samples from human biopsies confirmed the association between SIK1 and hypertrophic genes. These results suggest that salt intake could trigger events that lead to increased expression levels of genes associated with CH, independently of increases in BP and that SIK1 is a mediator of this process.

Since SIK1 can affect the transcription of genes that are associated with CH, the involvement of a hypertensive form of α-adducin and SIK isoforms in this process was examined. mRNA levels for SIK2, α-adducin, and several markers of CH were correlated in tissue biopsies obtained from human hearts. Evidence that SIK2 is critical for the development of CH in response to chronic high-salt diet was also obtained in mice with ablation of the *Sik2* gene. Increases in heart size upon high salt diet, occurred only in $Sik2^{+/+}$ but not in $Sik2^{-/-}$ mice. The presence of a hypertensive variant of α-adducin in Milan rats (before they become hypertensive) was associated with elevated transcription factors and genes associated with CH. Thus, we concluded that CH, triggered by salt intake or the presence of a genetic variant of α-adducin, requires SIK2 and is independent of elevated BP.

Taken together, this thesis describes how the SIK network could affect BP regulation and independently of BP mediate the effect of salt on the development of CH.

LIST OF PUBLICATIONS

This thesis is based on the following research articles, referred to in the text by their corresponding roman numerals (I-III).

I. **Popov S**, Silveira A, Wagsater D, Takemori H, Oguro R, Matsumoto S, Sugimoto K, Kamide K, Hirose T, Satoh M, Metoki H, Kikuya M, Ohkubo T, Katsuya T, Rakugi H, Imai Y, Sanchez F, Leosdottir M, Syvanen AC, Hamsten A, Melander O, Bertorello AM. Salt-inducible kinase 1 influences Na(+),K(+)-ATPase activity in vascular smooth muscle cells and associates with variations in blood pressure. *J Hypertens.* 2011 Dec;29(12):2395-403

II. **Popov S**, Venetsanou K, Chedrese PJ, Pinto V, Takemori H, Franco-CerecedaA, Eriksson P, Mochizuki N, Soares-da-Silva P, Bertorello AM. Increases in intracellular sodium activate transcription and gene expression via the salt-inducible kinase 1 network in an atrial myocyte cell line. *Am J Physiol Heart Circ Physiol. 2012 Jul;303(1):H57-65*

III. **Sergej Popov**, Hiroshi Takemori, Laura Brion, Per Eriksson, Lucia Torielli, Patricia Ferrari, Naoki Mochizuki, Giuseppe Bianchi, Alejandro M. Bertorello. Genetic predisposition or high salt intake cause cardiac hypertrophy through activation of SIK2 independently of blood pressure levels. *Manuscript*

.

CONTENTS

LIST OF ABBREVIATIONS

A7r5	Rat aortic cell line
ACTA1	Skeletal actin
AMPK	Adenosine monophosphate activated protein kinase
ATCC	American type culture collection
BNP	Brain natriuretic peptide
BP	Blood pressure
CaMK	Calmodulin kinase
cDNA	Complementary DNA
CH	Cardiac hypertrophy
CNS	Central nervous system
DBP	Diastolic blood pressure
EDTA	Ethylenediamine tetraacetic acid
EGTA	Ethylene glycol tetraacetic acid
GST	Glutatione-S-transferase
HA	Hemagglutinin epitope
HDAC	Histone deacetylase
HL-1	Mouse heart atrium cell line
LV	Left ventricle
MDC-CC	Malmoe diet and cancer study cardiovascular cohort
MEF2	Myocyte enhancer factor 2
MHC	Myosin heavy chain
MHS	Milan hypertensive strain
MNS	Milan normotensive strain
Mon	Monensin
MPP	Malmoe preventive project
mRNA	messenger RNA
MYH7	Myosin heavy chain gene
NCX	Sodium-calcium exchanger
NFAT	Nuclear factor of activated t-cells
NK	Na^+/K^+-ATPase
NPPB	Brain natriuretic peptide gene
OLF	Ouabain-like factor
RAAS	Renin angiotensin-aldosterone system
RNA	Ribonucleic acid
RPLP0	60S acidic ribosomal protein P0
SBP	Systolic blood pressure
scRNA	scramble RNA
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
siRNA	Small interfering RNA
SIK	Salt-inducible kinase
SkA	Skeletal actin
VSMC	Vascular smooth muscle cells
WB	Western blot
WT	Wild type

1 INTRODUCTION

Hypertension, a raise in arterial blood pressure (BP), remains a big worldwide problem and is a major factor contributing to the development of cardiovascular diseases such as cardiac hypertrophy (CH). Despite recent advances in understanding risk factors and pathophysiology behind this condition, hypertension prevalence continues to increase. From data based on the participants from the Framingham Heart Study, untreated hypertension has been estimated to shorten the life expectancy with around 5 years (Franco, Peeters et al. 2005) for both men and women. As many as 1.56 billion people worldwide may be affected by the year 2025 (2007) and developing countries in Africa and Asia and western countries with fully developed healthcare systems are affected.

Around 95% (Cowley 2006) of all people having high BP have unknown cause of the disease which is classified as essential (primary) hypertension. Secondary causes, such as renal failure, monogenic causes or aldosteronism, among others, comprise the secondary form of hypertension (Carretero and Oparil 2000). Lifestyle factors such as alcohol, tobacco consumption, a salt-rich diet, processed or fatty foods in combination with environmental and genetic factors have been associated with the development of primary hypertension (Mullins, Bailey et al. 2006). In developed countries, physical inactivity and high salt diet are thought to be the among biggest contributors to development of primary hypertension (Carretero and Oparil 2000). Despite the availability of a variety of antihypertensive drugs, the prevalence of hypertension increases even in the developed countries where the drug cost is rarely a problem.

1.1 CARDIAC HYPERTROPHY

One of the major complications of sustained elevated BP is CH, which refers to the enlargement of the myocardium. At the cellular level, this is characterized by increased activity of a variety of transcription factors, increased transcription of genes associated with CH, altered gene expression profile and increased protein synthesis which leads to larger cell size and eventually to CH. Pathological CH causes a change in heart geometry which leads to reduced cardiac function, fibrosis and could ultimately lead to heart failure. The intracellular signaling pathways leading to development of hypertrophy remain poorly understood.

1.2 HYPERTENSION

The most common definition of hypertension is when after two consecutive visits to a doctor or after two subsequent measurements the systolic blood pressure is ≥140 mm Hg and/or diastolic blood pressure is ≥90 mm Hg (Carretero and Oparil 2000). In western countries, systolic blood pressure (SBP) tends to rise with age leading to a prevalence of systolic hypertension in industrialized countries (Carretero and Oparil 2000). BP tends to vary among ethnical groups. For instance in US, black subjects have the highest prevalence of high BP while the Mexican population has the lowest. Socioeconomic factors and lifestyle also affect the prevalence and mortality rate of people affected with high BP. Other factors, such as gender and geographic location also have effect on BP prevalence.

Idiopathic, essential and primary hypertension, are the terms for same type of hypertension, when there is no known cause to high BP. This is the most common type of hypertension and accounts for around 95% of all the cases worldwide. Secondary hypertension accounts for the remaining cases and is caused by diseases such as renal failure, aldosteronism, renovascular disease and endocrine diseases.
Even though essential hypertension is generally said to have unknown causes there are a number of factors affecting development of high BP. These factors are high alcohol intake, high sodium intake, obesity and insulin resistance, low potassium or calcium intakes and genetic predisposition. These factors by themselves or in combination can influence the development of high BP. There have been some studies that point to weak associations between polymorphisms in certain genes, such as α-adducin (Manunta, Burnier et al. 1999) and essential hypertension.

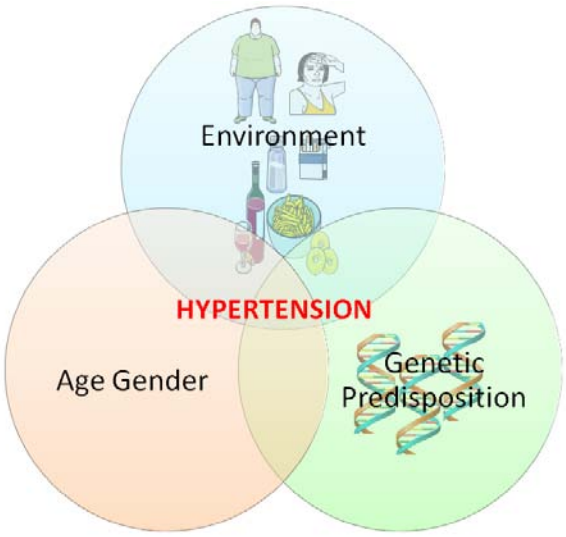

Figure 1. A model of the essential hypertension. Genetic predisposition together with age, gender and various environmental factors affect the development of high blood pressure.

BP is mainly dependent on two factors: total peripheral resistance and cardiac output (Cowley 2006). Cardiac output is in turn dependent of heart rate, heart stroke volume extracellular fluid volume, whilst peripheral resistance is dependent on vascular function and structural changes in arteries. Hypertension has severe effects on several human organs. In the heart the high pressure causes increased workload which could lead to hypertrophy, and ultimately to heart failure if left untreated. In the brain the increased pressure damages blood vessels causing brain infarction and stroke which causes several brain disorders and death. In kidneys the pressure has various effects such as nephrosclerosis (Marin, Gorostidi et al. 2005).

1.3 KIDNEYS AND HYPERTENSION

Kidneys play a major role in BP regulation. When the blood volume increases and the vascular resistance remains the same the systemic pressure will also increase. The increased pressure will cause kidneys to excrete more fluid and salt. The blood volume decreases causing the heart to pump a lesser amount of blood and the arterial pressure decreases. In contrast to chemoreceptors, baroreceptors and CNS systems that affect short term BP, the fluid based-system controls long term BP levels. Even though increased fluid intake affects the blood volume, studies have shown that in fact increased sodium intake is the major contributing factor to increased BP because an excess of water will be excreted rapidly while salt is harder to excrete. The increased sodium levels lead to increased water intake which increase systemic BP. Thus, hypertension arises ultimately after kidneys are unable to cope with sodium excretion (Guyton 1991).

While renal diseases can cause secondary hypertension, primary hypertension can causes secondary renal diseases. Some of the diseases caused by essential hypertension are nephrosclerosis and hypertensive kidney disease (Marin, Gorostidi et al. 2005). Hypertension is second only to diabetic nephropathy as a major cause of end stage renal disease (Bidani and Griffin 2002).

1.4 GENETIC PREDISPOSITION

As mentioned previously, 95% of the hypertensive cases are classified as essential or primary hypertension. It is estimated that essential hypertension has a heritability of 30-50%. Therefore it is of importance to find the genes involved in hypertension development to help to improve treatment strategies, understand the biochemical pathways and recognize individuals that risk developing this disease. So far, very few genetic factors that contribute to the development of primary hypertension have been identified. The simplest approach to define genes that are affecting BP is by studying families or relatives that are affected with hypertension. A detailed linkage analysis can

then sometimes reveal the specific locus or even a gene that is affecting the BP. In this type of cases when simple Mendelian form is affecting BP, it is mostly due to rare genetic disorders (secondary hypertension). Some examples being: Liddle's syndrome when a genetic mutation in the epithelial sodium channel leads to an abnormal re-absorption of sodium and loss of potassium which leads to hypertension (Lifton, Gharavi et al. 2001). Another example is the mutation in peroxisome proliferator-activated receptor γ (PPARG) which leads to insulin resistance diabetes and hypertension (Barroso, Gurnell et al. 1999). These, and a few other mutations giving rise to different syndromes and diseases that also give rise to high BP, account for a very small percentage of the cases of hypertension. Interestingly, most of the mutations causing high BP derived from Mendelian inheritance have been found to, in one way or another, be associated with salt re-absorption.

For the essential hypertension, contribution from one single gene is not enough to affect the development of hypertension. For this multifactorial disease, small contributions from several genes, often coupled with different environmental factors and random genetic variations within these genes, lead to high BP development. The effect of the genes contributing to elevated pressure could also be modified by other unrelated genes. Genome-wide association studies (GWAS) and candidate gene studies have been far less successful, often incomplete, or contradictory when identifying genes or genetic markers that affect development of hypertension.
In candidate gene studies a large number of different genotypes are screened at chromosome loci of proteins in pathways that are implicated in BP regulation, such as the renin-angiotensin-aldosterone (RAAS) system. In GWAS, SNPs are distributed throughout the genome, without prior knowledge of the biology of the disease.

Two different studies have reported a linkage of essential hypertension to chromosomes 2 and 17 (Hsueh, Mitchell et al. 2000; Levy, DeStefano et al. 2000) A recent GWAS involving over 200,000 individuals of European descent (Ehret, Munroe et al.) revealed new loci associated with BP and confirmed some of previously identified signals from GWAS. However, such studies are specific for certain populations or the significance threshold for the linkage between genes and hypertension is not reached. Because in different GWAS there has not been any particular locus interval repeatedly linked to hypertension there might be several loci on different chromosomes that in combination together with environmental factors and lifestyle add up small effects on BP ultimately affecting the development of hypertension.

1.5 HORMONES AND HYPERTENSION

CNS, chemoreceptors, baroreceptors and RAAS system are important in terms of BP regulation. Whereas CNS, chemoreceptors and baroreceptors are involved in short-term responses (within seconds) to changes in the BP, RAAS is activated within minutes of the decreased BP (Guyton 1991). Increased salt intake causes increased thirst and higher intake of fluids. This will then affect the RAAS system. RAAS is the hormone system that plays a critical role in regulation of BP in human body. In response to lower BP renin is released into blood circulation from juxtaglomerular cells in the kidney. Circulating renin cleaves angiotensinogen that is produced by the liver to angiotensin I (AngI). AngI is then converted to angiotensin II (AngII) by angiotensin converting enzyme (ACE) released from capillaries of the lung. AngII has various effects in the human body most of which are exerted by binding to angiotensin receptor type I (AT1), leading to increased sodium re-absorption in kidneys, vasoconstriction and affecting the glomerular filtration rate which increases BP (Unger 2002). In addition, AngII stimulates release of the hormone aldosterone which also enhances distal tubule sodium re-absorption (Weir and Dzau 1999). Current treatment of hypertension is targeted to decreasing the vascular tone using ACE inhibitors as well as AT1 receptor blockers, diuretics, β-blockers and calcium channel blockers. The most common method of treatment is to use two or more drugs in combination to reduce BP (Paulis and Unger), however there is no one-for-all drug strategy and the type of treatment often depends on the BP and whether the patient has a specific disease.

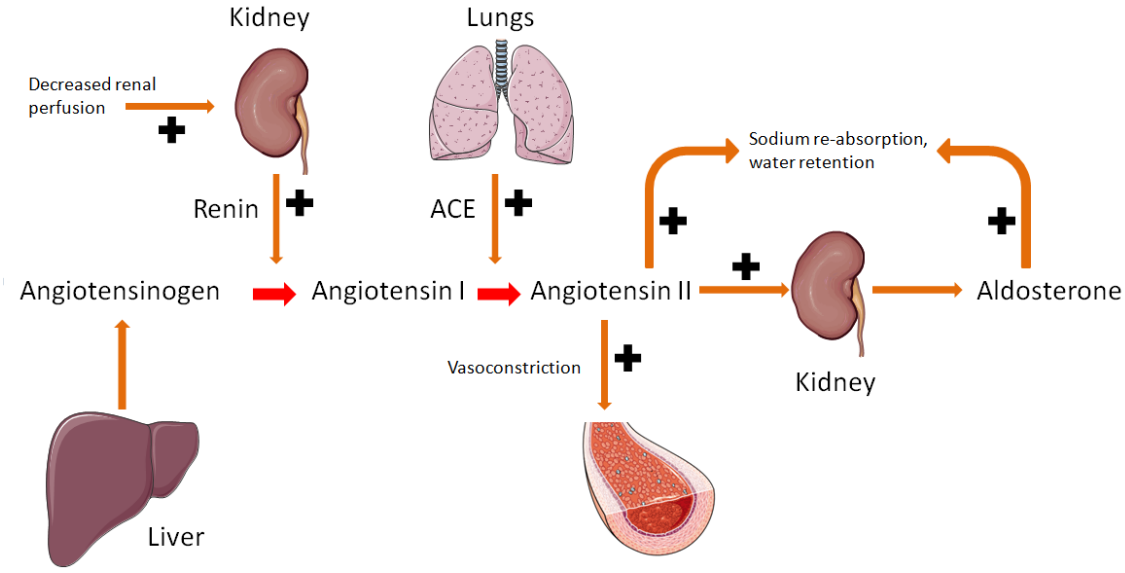

Figure 2. The renin-angiotensin-aldosterone system (RAAS). (**+**) denotes a stimulatory effect

Increased sodium intake leads to increased release of aldosterone and also stimulates production of endogenous ouabain-like factors (OLF). These OLF target the α2 isoform of the Na^+/K^+-ATPase (NK) pumps in the vasculature and in the myocardium, inhibit

NK, thereby increasing the vascular tone and myocardium contraction which in turn increases BP. This has been shown in rats where low levels of OLF induced hypertension. Nanomolar concentrations of circulating endogenous OLF has been shown to induce release of AngII in spontaneously hypertensive rats at the same time as it induces the release of nitric oxide which has the opposite effect of AngII (Dong, Komiyama et al. 2004; Padilha, Rossoni et al. 2004). Recently a novel sodium sensing network has been discovered (Sjostrom, Stenstrom et al. 2007). When concentrations of sodium raise, a salt-inducible kinase 1 (SIK1) is increased in activity. The increased SIK1 activity controls the activity of NK which are involved in sodium homeostasis. Interestingly, cells that express the hypertensive form of the α-adducin that causes Milan hypertensive rats become spontaneously hypertensive, have also increased SIK1 activity (Stenstrom, Takemori et al. 2009). With the current advances in OLF and sodium signaling research, targeting ouabain-induced intracellular signaling and SIK1 activity might be effective treatment of hypertension.

1.6 LIFESTYLE AND ENVIRONMENTAL RISK FACTORS

A number of lifestyle and environmental factors influence the development of hypertension. Alcohol, smoking, obesity, diet and stress are all thought to affect BP.

1.6.1 Alcohol intake

Alcohol elevates both the acute and the chronic BP. Even though the mechanism by which alcohol affects BP is not well known, results of studies involving different human populations have been consistent and show that alcohol does affect BP. Potential mechanisms by which alcohol could affect the BP include the modulation of RAAS system, the heart rate and the vascular smooth system (Beilin, Puddey et al. 1996; Klatsky 1996). It has been shown that refraining from drinking alcohol every day lowers BP significantly (Kawano).

1.6.2 Smoking

It has yet to be established how smoking affects BP. It is has been shown that smoking increases acute BP and BP decreases among smokers who have not been smoking for a period of time (Minami, Ishimitsu et al. 1999). In contrast, in non-clinical samples non-smokers and former smokers have significantly higher BP levels than current smokers (Lee, Ha et al. 2001; Okubo, Suwazono et al. 2004). However, the general recommendation for smokers suffering from hypertension is to quit smoking to minimize the CVD mortality risk.

1.6.3 Obesity

Both clinical and animal studies point to a strong association between obesity and hypertension (Hall 2003). Increases in fat mass, especially increases in visceral adipose tissue, lead to increased release of adipocytokines such as endothelin, IL-6, renin, non-esterified fatty acids and aldosterone that influence development of hypertension (Tilg and Moschen 2006; Katagiri, Yamada et al. 2007). Although exact mechanisms between obesity and hypertension are not very well understood, obesity induces several systems that lead to development of hypertension. Obese individuals have been shown to have an increased sympathetic nervous system (SNS) activity (Scherrer, Randin et al. 1994; Huggett, Burns et al. 2004). Increase in SNS activity indirectly, via activation of RAAS system, causes elevated heart rate, increased BP levels and increased renal sodium re-absorption. Moreover, high sodium intake that often concurs with high calorie intake and obesity further enhances the effect on BP.

1.6.4 Stress

In a review by Gerin and Lovallo (Lovallo and Gerin 2003) psychological stress was defined as "events that challenge the homeostasis of the organism because of their perceived threat value, regardless of potential for physical harm". This includes stress from daily job, natural catastrophic events such as earthquakes and tornados, stress of speaking publicly or stress derived from fear visiting a doctor (white coat hypertension). This acute stress causes transient elevations in BP; however it is yet unclear whether this could lead to permanent increases in BP. The European Society of Hypertension and The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, both state that 'Lifestyle measures should be instituted whenever appropriate in all patients, including subjects with high normal blood pressure and patients who require drug treatment (2003; Chobanian, Bakris et al. 2003) however, in these recommendations stress handling is not included.

It is important to note that many of the environmental factors often are additive, such as obesity and alcohol or obesity and smoking. Furthermore, environmental factors interact with the genetic factors contributing to the overall chance to develop hypertension. Even though in rat models a modification towards healthier lifestyle and a healthier diet were not shown to improve the mortality rates, the decrease of the risk factors may lead to a lower BP and less hypertensive drug usage avoiding the side-effects some drugs might induce.

1.7 SODIUM AND HYPERTENSION

Salt has only become an important ingredient in our diet over the recent 10.000 years. Our ancestors had a diet consisting of less salt intake than 1g/day which means that currently we are genetically programmed to handle no more than that amount of salt as opposed to today's worldwide average consumption of 100mmol/day (Brown, Tzoulaki et al. 2009). High salt intake has been implicated in the development of hypertension and hypertrophy; however there is great inter-individual variation in response to salt intake.

It has been shown that hypertension is strongly associated with salt consumption. In modern society the dietary salt intake has increased considerably compared to for instance the consumption during the Stone Age. Some tribes in Amazonas who live under the same conditions as Stone Age humans have a rich potassium and a minimum sodium diet which is approximately 20 times less than the average intake of sodium by a person living in a modern developed country. In these tribes there is no hypertension observed, suggesting the importance of sodium intake for the development of high BP. Epidemiological studies have shown that average sodium intake in developed countries should be around half of what it is now. Generally, in populations with low sodium intake the prevalence of hypertension is much lower than in populations with high salt diet.

Today the average salt intake is more than 3 times of the recommended daily intake. Numerous studies, both regional and worldwide, report that there is a correlation between salt excretion and BP. The INTERSALT (1988; Stamler, Rose et al. 1991) study involving 52 different centers and 10079 individuals, found a significant relationship between lower salt intake and a lower systolic BP as well as an association between the BP and sodium excretion after adjustment for age. Another worldwide study, Cardiovascular diseases and alimentary comparison (CARDIAC) (Yamori, Nara et al. 1990), found a significant positive correlation between both systolic- and diastolic blood pressures and 24h sodium excretion in men. A recent meta-analysis of 167 different studies that estimated the effects of low and high sodium intake on BP and the different circulating hormones and lipids was performed. In this analysis it was found that across the studies in normotensive Caucasians the lower sodium intake caused a BP decrease of only 1%, while in hypertensive Caucasians the short term lowering of sodium intake decreased the BP with 2-2.5% (Graudal, Hubeck-Graudal et al.). Some studies suggest a favorable effect of reduced sodium intake on cardiovascular outcomes. For instance, a study examining the long-term effects of sodium reduction reported a significantly lower amount of cardiovascular events in the group with sodium reduction in the diet compared to the non-intervention group (Cook, Cutler et al. 2007).

2 COMPLICATIONS OF CHRONIC HYPERTENSION

2.1 RENAL

Hypertension affects the detrimental progression of most end stage renal diseases (ESRD) (1998). While hypertension can cause renal diseases, renal diseases can also cause hypertension. Kidneys of spontaneously hypertensive rats (SHR) transplanted to normotensive recipient rats cause elevated BP and kidneys of normotensive donor rats lower BP in SHR rats (Curtis, Luke et al. 1983; Grisk and Rettig 2001). According to Guyton (Hall, Guyton et al. 1996) hypertension occurs as a result of the kidneys inability to cope with increased sodium intake, meaning that the BP increases in response to increased concentrations of sodium.

Because kidneys control most of the sodium homeostasis in humans, they also control the BP regulation. BP has been shown to be associated with risk of renal failure, as reported in three independent cohort studies (Klag, Whelton et al. 1996; Tozawa, Iseki et al. 2003; Hsu, McCulloch et al. 2005). In two of the studies which showed a relationship between increased BP and risk of ESRD, renal function was not examined. Hsu et al (Hsu, McCulloch et al. 2005) also reported association between BP and ESRD, while performing renal function studies. Even small increases in BP were significantly associated with the prevalence of ESRD.

The severity of damage inflicted by high BP on kidneys depends on several factors: the BP level, how much of the BP load is transmitted to the kidneys and structural characteristics of the renal microvasculature which vary between individuals (depending on genetic predisposition and previous kidney damage) (Beilin, Goldby et al. 1977; Neuringer and Brenner 1993; Bidani and Griffin 2002). To avoid complications that may arise in kidney due to high pressure, treatment of hypertension involves targeting the RAAS system to reduce BP and to avoid the resulting kidney damage (Fogo 2000; Taal and Brenner 2001). This treatment includes ACE blockers and ATII receptor antagonists, among others. Because drugs targeted towards RAAS system often counteract the natriuresis by lowering the BP, and because salt sensitivity increases in damaged kidneys (Johnson, Gordon et al. 1999) a therapy involving drugs such as thiazides is sometimes also used in combination with RAAS system inhibitors.

2.2 CARDIAC

Cardiac complications arising from chronic hypertension are collectively called hypertensive heart diseases (HHD) which includes conditions such as coronary artery disease, systolic and diastolic myocardium dysfunctions, arrhythmia and left ventricular hypertrophy, among others. According to a meta-analysis of several prospective studies, the contribution of hypertension to cardiovascular diseases is greater than any other risk factor and even small increases in BP double the risk of ischemic heart disease and stroke in people between 40-90 years of age (Lewington, Clarke et al. 2002). Genetic predisposition and hypertension are the two of the major factors contributing to the development of left ventricular hypertrophy (Levy, Anderson et al. 1988; Galderisi, Celentano et al. 1993; Arnett, Hong et al. 2001).

As with any other muscle, increased load on the heart leads to muscle hypertrophy. However one should distinguish physiological hypertrophy from pathological hypertrophy. Physiological hypertrophy occurs after excessive exercise or pregnancy. This form of hypertrophy is reversible and is characterized by enlargement of the left ventricular chamber and a uniform increase in ventricular wall and septum. The myocyte length increases more than the width by addition of sarcomeres in the middle and in the periphery of the myocytes. The proportions between the chamber increase and wall increase remain the same and the normal cardiac structure is maintained. This does not lead to any abnormal heart function and is a natural adaptation of the heart to cope with increased work load. Pathological hypertrophy is not reversible unless treated and is characterized by a thickening of the ventricular wall and septum along with remodeling and a decreased size of the left ventricular chamber. The width of the myocytes is increased more than the length (Heineke and Molkentin 2006).

To increase in size, myocytes have to change the quantitative and qualitative gene expression patterns induced by activation of various transcription factors which leads to increased protein synthesis. The precise molecular signaling by which hypertension and increased mechanical load leads to activation of various intracellular mechanisms and eventually to development of CH is not well understood. However, several signaling pathways have been suggested. The general consensus is that some of the mechanisms leading to increased heart size differ between the pathological and physiological hypertrophy.

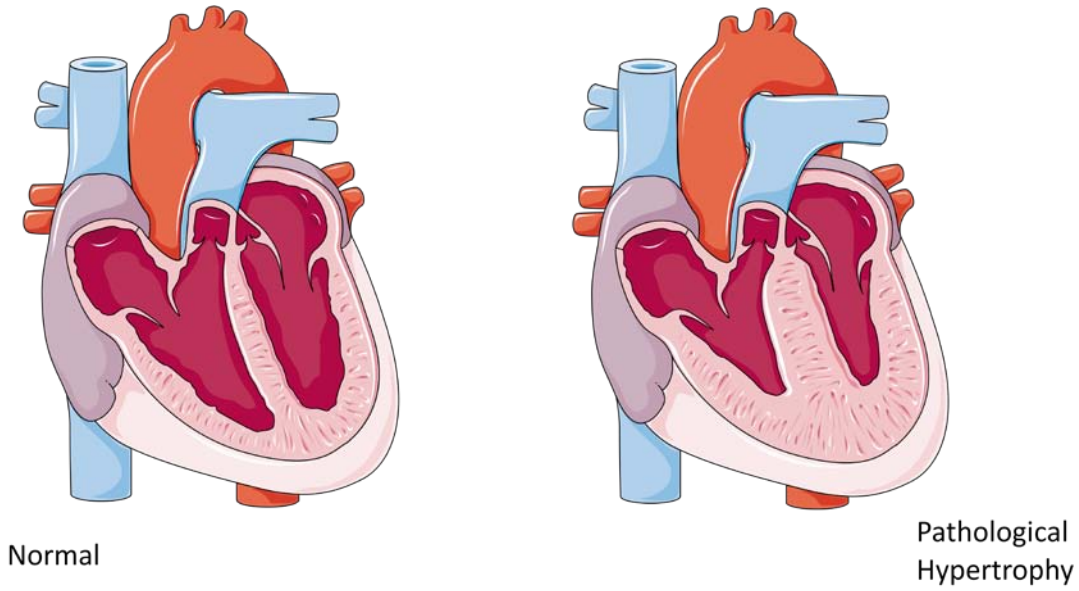

Figure 3. Pathological cardiac hypertrophy. The left ventricle wall and septum are thicker compared to the normal heart because of non-uniform addition of sarcomeres.

Mechanical stimuli can initiate increased protein synthesis (Ruwhof and van der Laarse 2000). This mechanical stress can be accompanied by increased concentration of circulating humoral factors (Ruwhof and van der Laarse 2000) although mechanical stress alone could also affect growth of the cardiomyocytes (Cooper, Kent et al. 1985). This has been proven when blockage of adrenal receptors gave an increased rate of protein synthesis (Oi, Haneda et al. 1999) or when isolated cardiomyocytes under increased load altered the rate of protein synthesis (Kira, Kochel et al. 1984). It has also been shown that stretching cardiomyocytes altered the gene expression profile without involvement of humoral factors (Komuro, Kaida et al. 1990; Sadoshima, Jahn et al. 1992; Kira, Nakaoka et al. 1994).

There are several circulating factors that can initiate growth of cardiomyocytes at the cell membrane, such as transforming growth factor-β (TGF-β) AngII, endothelin-1, catecholamines (noradrenalin and adrenaline) and insulin growth-like factor-1 (IGF-1) (Ruwhof and van der Laarse 2000) (the latter is implicated in the development of physiological but not pathological hypertrophy). The release of these humoral factors can be triggered by stretching cardiomyocytes, by hemodynamic load or in an autocrine fashion. These circulating factors interact with specific target receptors on the cell surface that conduct the signal via different intracellular pathways to transcription factors which alter the gene expression profile and affect the rate of gene transcription. Transcription factors may act alone or interact with one another to co-activate the target genes (Akazawa and Komuro 2003). The molecular signaling which leads to activation of transcription factors in response to either humoral factors or cell stretching is

complex and several intermediate proteins and molecular pathways are involved (Heineke and Molkentin 2006).

Two of the transcription factors that have been implicated in development of hypertrophy are myocyte enhancer factor 2 (MEF2) and nuclear factor of activated T-cells (NFAT). As the name suggests, NFAT is a mediator in the t-cell activation, is present in a variety of tissues and has five isoforms: NFATc1-c5. Later it was found that it also acts as a transcription factor inducing the expression of genes associated with hypertrophy. In response to dephosphorylation at the N-terminus by a variety of molecules such as calcineurin, NFAT translocates from the cytoplasm to the nucleus where it initiates the transcription. NFAT seems to be involved in the regulation of pathological but not physiological hypertrophy. Transgenic mice that express activated NFAT3 have been shown to develop hypertrophy and heart failure (Molkentin, Lu et al. 1998). When transgenic mice expressing a luciferase NFAT reporter were subjected to both physiological (training) stimuli and pathological (pressure overload) stimuli the reporter activity was upregulated only in the mice exposed to pathological stimulus (Wilkins, Dai et al. 2004). In addition, inhibition of NFAT *in vitro* leads to inhibition of hypertrophy induced by endothelin-1 and calcineurin (van Rooij, Doevendans et al. 2002). NFAT is one of the proteins which can interact with other transcription factors in order to affect gene transcription. It has been shown that in response to AngII NFAT binds to GATA4 to promote *BNP* gene transcription (Morimoto, Hasegawa et al. 2001). Moreover, NFAT interacts with MEF2 and enhances MEF2 activity in response to calcineurin (van Oort, van Rooij et al. 2006).

MEF2 has a variety of functions in the cell including: muscle generation, tumorigenesis and neuronal cell development (Potthoff and Olson 2007). As with NFAT, MEF2 is also implicated in the development of CH and is also able to interact with GATA4 to enhance transcription of its target genes in a synergistic fashion (Morin, Charron et al. 2000). MEF2 is controlled by its association with class II histone deacetylases (HDAC). Under basal conditions MEF2 activity is inhibited by direct binding of HDAC to MEF2 in the nucleus. In response to extracellular stimuli and the subsequent increased calcium concentrations, class II HDAC dissociate from MEF2. There are a number of kinases that are capable of phosphorylating class II HDAC. SIK1 has been shown to be the kinase which phosphorylates class II HDAC. In response to catecholamines, CREB enhances the expression of SIK1 which then can affect the activity of MEF2 by phosphorylating class II HDAC (Berdeaux, Goebel et al. 2007).

Most models of CH are associated with up-regulation of genes that are now considered to be hypertrophic marker genes. These genes include β-myosin heavy chain (β-MHC), brain natriuretic peptide (BNP), atrial natriuretic peptide (ANP) and skeletal actin

(SkA). As a part of the heart adaptation process, the myosin isoforms undergo a switch from the more energy demanding α-MHC to the slower and energy efficient β-MHC isoform. The increased expression of the β-MHC have been found in most types of hypertension (Palmer 2005). However the switching to the slower isoform eventually causes systolic dysfunction. In addition, hypertrophy could lead to a down-regulation of certain genes, which also commonly used as markers of CH, namely α-MHC and sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) (McMullen, Shioi et al. 2003).

3 SALT-INDUCIBLE KINASE

3.1 THE SIK PROTEIN

Salt-inducible kinases (SIKs) are a family of serine/threonine kinases. Of these, the SIK1 is the one most intensively studied. SIK1 is a homolog of sucrose non-fermenting like kinase 1 (snf1lk) that has been found in a screen for kinases during mouse cardiogenesis (Ruiz, Conlon et al. 1994). It was found to be up-regulated in the developing heart suggesting involvement in the process of cardiogenesis. Snf1lk is 50% identical to the protein SNF-1 found in yeast which is activated in response to nutrient stress. Later, a homolog of snf1lk was found in adrenal glands of rats that were fed high salt diet (Lin, Takemori et al. 2001).

In humans, SIK1 gene is located on chromosome 21, while the two other genes SIK2 and SIK3 are located on chromosome 11 (Katoh, Takemori et al. 2004). SIK1 and its two isoforms are 776 amino acids, 931 amino acids and 1263 amino acids long respectively. The three kinases, all have a highly conserved serine/threonine kinase domain at the N-terminal end, a SNF-1 domain in the middle and a site of potential serine phosphorylation at the C-terminal end. Of the three isoforms, SIK1 is found ubiquitously in human cells while SIK2 and SIK3 are found in specific tissues only. SIK homologs have been found in rats, mice and recently in chickens (Xia, Zhang et al. 2000) and *drosophila melanogaster* (Adams, Celniker et al. 2000). A homolog to SIK, SOS2 is also found in plants where it is involved in responses to changes in salt concentration (Bertorello and Zhu 2009). The highly conserved forms of this kinase between species, suggest the importance of this protein. Indeed SIKs have been implicated in various intracellular pathways that are important for cell growth and survivability.

3.2 STEROIDOGENESIS AND ADIPOGENESIS

SIK1 is present in the adrenal cortex and its expression in cells is stimulated by increases in adrenocorticotropin (ACTH) (Jefcoate, Lee et al.), proceeding an increase in steroidogenic proteins. In addition, SIK1 was found to repress steroidogenic gene transcription (Okamoto, Takemori et al. 2004). ATCH treatment of mouse adrenocortical cells stimulates translocation of SIK1 from nucleus to cytoplasm (Okamoto, Takemori et al. 2004). This suggests that SIK1 might be involved in steroidogenesis in the adrenal cortex. SIK2 isoform expression has been found to be increased in adipocytes of type 2 diabetic mice (Horike, Takemori et al. 2003). During

normal conditions, insulin binding to the cell surface activates a tyrosine phosphorylation cascade leading to insulin receptor substrate-1 (IRS-1) activation and a lipogenic response in cultured 3T3-L1 adipocyte cells.

3.3 ISCHEMIA

Cerebral (brain) ischemia refers to insufficient blood flow to the brain which leads to oxygen deprivation causes energy starvation, decreased metabolic rates and death of cerebral tissue (infarction). Recently it has been established that SIK2 is involved in the protective molecular signaling pathway that is activated by glucose and oxygen starvation in cortical neuronal cell cultures (Cheng, Uchida et al.). Mice lacking SIK2 have been shown to have a higher neuronal survival rate compared to the wild type mice in response to oxygen and glucose deprivation (Cheng, Uchida et al.). In the proposed intracellular pathway, CREB acts, via transducer of regulated CREB activity (TORC) and SIK2, activating neuronal pro-survival genes (Gallo and Iadecola). Briefly, in response to oxygen and glucose deprivation calmodulin-dependent kinase 1 (CaMK1) phosphorylates SIK2 at Thr484 residue which leads to SIK2 degradation. Lack of SIK2 leads to dephosphorylation of TORC causing nuclear import of TORC where it activates CREB. CREB then increases the expression of pro-survival genes (Sasaki, Takemori et al.). Therefore, the SIK2-TORC-CREB pathway could be a potential target for protection of brain against ischemia and stroke.

3.4 CELL SODIUM HANDLING

Most cells are highly permeable to water and must maintain a regulated cell volume to be able to maintain the intact structure, avoiding shrinkage or swelling, in order to function normally. Very small ions and charged molecules will diffuse into the cell freely but large charged molecules will not. The negative charge inside the cells is caused by the negatively charged organic substances such as proteins and aminoacids. This will create an uneven distribution of negatively charged molecules and will cause an influx of ions and water into the cell. Cells maintain a membrane potential by keeping higher concentrations of sodium in the extracellular compartment while potassium is stored in the intracellular space. This balance is maintained by the NK pump. NK utilizes ATP to actively transport three sodium ions outwards across the membrane and at the same time transport two potassium ions from extracellular compartment inward against the ion gradient. The water that follows sodium out of the cell also helps the cell to prevent swelling.

Besides being used in the vital cell volume regulation, sodium is vital to cell function as a signaling molecule in cardiac myocytes. It is involved in contraction, energy

metabolism and cardiac growth, as well as influencing the calcium signaling via the sodium-calcium exchanger (NCX). Because sodium is important in the development of hypertension and hypertrophy it is of importance to elucidate the precise molecular pathways involving sodium to be able to develop new therapeutic agents. The SIK network is involved in sodium regulation and signaling inside cells and in this thesis this network was the central focus, with the aim of better understanding the intracellular signaling that can cause hypertrophy and hypertension.

4 HYPOTHESIS

4.1 GENERAL AIM

The aim of this study was to determine whether SIK1 could be involved in the blood pressure regulation and to elucidate the importance of the salt-inducible network during the development of cardiac hypertrophy. More specifically, we set out to examine the involvement of the salt-inducible kinase in the signaling cascade leading to the development of hypertension and examined whether the different isoforms of salt-inducible kinase are implicated in the different intracellular mechanisms that can affect the development of cardiac hypertrophy independently of changes in BP.

4.2 SPECIFIC AIMS

4.2.1 Aim 1

To examine whether salt-inducible kinase 1 affects Na^+/K^+-ATPase activity in vascular smooth muscle cells and endothelial cells and whether genetic variation within SIK1 could affect blood pressure in humans.

4.2.2 Aim 2

To elucidate whether sodium could, via the salt-inducible kinase network affect the activation of transcription factors involved in the development of cardiac hypertrophy and whether sodium could affect myocyte growth independently of high blood pressure.

4.2.3 Aim 3

To determine whether salt and a genetic variation in the α-adducin gene are involved in the development of cardiac hypertrophy independently of sustained changes in blood pressure and whether salt-inducible kinase network mediates this effect.

5 MATERIALS AND METHODS

5.1 CELL CULTURES

The cardiac atrial cell line HL-1 was used as the experimental model for cardiac myocytes. HL-1 is a mouse atrial cell line that can be recovered from frozen stocks, be serially propagated in culture and still retain the biochemical and physiological properties of adult cardiomyocytes such as contraction and expression of cardiac specific markers. This cell line expresses the proteins found in adult cardiac myocytes such as a myosin heavy chain, skeletal actin, atrial natriuretic peptide and sarcomeric myosin (Claycomb, Lanson et al. 1998) among others. This cell line also expresses SIK1 and SIK2 isoforms (Popov, Venetsanou et al.). The cells were maintained at 37°C in an atmosphere of 5% CO_2 and 95% air and cultured in Claycomb medium (Claycomb, Lanson et al. 1998) (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 pre-coated with fibronectin [25 μg fibronectin (Sigma) in 2 ml of 0.02% gelatin (Sigma)].

The A7r5 cell line was used as the experimental model for smooth vascular muscle cells. This cell type derives from rat aorta smooth muscle and possesses the properties of vascular smooth muscle cells such as expressing the creatine phosphokinase muscle type and being able to generate action potentials at some stage of the development (Kimes and Brandt 1976). The cells were from ATCC (American Type Culture Collection, Manassas California, USA) and were maintained at 37°C in atmosphere of 5% CO_2 and 95% air and cultured in Dulbecco's modified Eagle's medium (Gibco, Carlsbad, California, USA) supplemented with 10% fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin.

5.2 LEFT VENTRICLE MASS DETERMINATION

Left ventricular mass in normotensive individuals has been found to be a predictor of development of hypertension (Devereux, de Simone et al. 1993). It is a strong predictor that reflects sodium intake and other pathophysiological factors affecting development of hypertension. Left ventricle mass was measured in 1792 participants of the MMP study who underwent echocardiography in the left lateral decubital position. All analyses were performed offline without any knowledge of the patients' clinical status and interobserver and intraobserver variability were tested by two readers

independently analyzing images from a random subsample of participants. Detailed instrument and measurement descriptions can be found in Article I Methods section.

5.3 SNP GENOTYPING

Four populations were genotyped for the human *SIK1* SNP rs3746951: Malmö Diet and Cancer study (15273 participants), Malmö Preventive Project (5144 participants), Stockholm cohort (1273 participants) and the Ohasama study (1162 participants). Illumina Infinium II Human 1M (Illumina, Inc., San Diego, California, USA) at SNP Technology Platform, Uppsala University was used for genotyping the Stockholm cohort and TaqMan methodology (assay C_3004267_10, Applied Biosystems) was used to genotype the Malmö and Ohasama cohorts.

5.4 DETERMINATION OF NA^+/K^+-ATPASE ACTIVITY

A7r5 VSMC grown in Petri dishes in standard A7r5 medium until 80% confluence were transfected with either the SIK1(^{15}Gly) or SIK1 (^{15}Ser) plasmids (7μg) for 8h in basal DMEM medium and allowed to express the proteins for 36h. Cells were then transferred into 24-well plates, with 70,000 cells/well for incubation overnight. Following this, the $^{86}Rb^+$transport assay was performed as described elsewhere (Pedemonte, Pressley et al. 1997; Efendiev, Bertorello et al. 2002) to measure the Na^+/K^+-ATPase activity.

5.5 IMMUNOHISTOCHEMISTRY

Immunohistochemistry was performed on abdominal aorta samples obtained from patients at Karolinska Hospital undergoing elective surgery. Briefly, tissues were fixed with formaldehyde and embedded in paraffin. Rabbit antihuman SIK1 antibody (1 : 50) was used to stain for SIK1. Tissue slices were incubated overnight at 4°C after deparaffinizing and antigen retrieval treatment with DIVA decloaker according to the manufacturer's instructions (Biocare Medical, Concord, California, USA). After washing in PBS, slides were incubated with a biotinylated horse antirabbit IgG (Dako A/S, Glostrup, Denmark) for 30 min at room temperature. Slides were then incubated with avidin–peroxidase complexes (ABC Elite Kit, Vector Labs, Burlingame, California, USA) for 30 min, followed by visualization with 3,30-diaminobenzidine tetrahydrochloride (Vector Labs). Isotypic rabbit IgG (Abcam, Cambridge, UK), used in the same concentration as for anti-SIK1 antibody, was used as a negative control for SIK1 staining. Slides were finally counterstained with Mayer's hematoxylin

5.6 TAQMAN QUANTITATIVE REAL TIME PCR

Total cellular RNA was isolated 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) and total cDNA was synthesized from 0.5µg RNA in a polymerase reaction using RevertAid H Minus M-MuLV reverse transcriptase (Fermentas, Life Science, Vilnius, Lithuania). All assays and reagents for real-time quantitative PCR were obtained from Applied Biosystems (Foster City, CA). All assays were performed using the ABI Prism 7000 sequence detection system according to the manufacturer's protocol, using five-point standard curves generated from 10-fold dilutions of purified PCR products. The results were expressed in arbitrary units or as percent change of units related to the levels of RPLPO mRNA expression for normalization.

5.7 LUCIFERASE ASSAY

Luciferase activity was determined in HL-1 cells transfected with NFAT-Luc vector (Clontech, Laboratories Inc., Palo Alto, CA), MEF2-Luc vector (Shin, Seoh et al. 1999), BNP-Luc vector (obtained from Professor David Gardner), β-MHC-Luc vector (originated from Dr Robert MacLellan) in combination with either SIK1-WT (Sjostrom, Stenstrom et al. 2007), SIK1-K56M (Sjostrom, Stenstrom et al. 2007), SIK1-T322A (Sjostrom, Stenstrom et al. 2007), α-adducins (G460/S586 normotensive, W460/C586 hypertensive) (Efendiev, Krmar et al. 2004), SIK2-WT (Horike, Takemori et al. 2003), SIK2-K49M (Horike, Takemori et al. 2003), HDAC-WT (Takemori, Katoh Hashimoto et al. 2009), HDAC (S259A) (Takemori, Katoh Hashimoto et al. 2009) or pRL-TK Renilla (Promega). Cells were plated into 24-well plates (70,000-100,000 cells/well) and transient transfections were performed using lipofection method (LipofectAMINE 2000, Invitrogen) according to the manufacturers' instructions. After incubations according to desired treatments and timing, the cell medium was removed and the reaction was stopped by washing the cells twice using ice-cold PBS and dissolved in 100 µl of luciferase assay buffer. The cell lysates were vortexed for 30 sec and incubated at –20 ºC for 5 min. Cells were further disrupted on a shaker for 30 min. Cell lysates (20 µl) were mixed with 100 µl of Luciferase Assay reagent (Promega, Madison, WI, USA) and the light produced was measured in a Luminometer (Turner Designs, Sunnyvale, CA, USA). For Renilla normalization Stop & Glo® Reagent (Promega, Madison, WI, USA) was added and the light emitted by Renilla luciferase was measured. Luciferase activity was expressed as the ratio between the light emitted by the luciferase-tagged plasmid and Renilla. Luciferase activity was also expressed as percentage change of the ratio between the light produced and the

protein concentration in the sample (RLU/mg of protein). Protein concentration in the cell lysates was determined using a commercial dye reagent (Bradford, BioRad).

5.8 GENE EXPRESSION DETERMINATION FROM HUMAN BIOPSIES AND ANIMAL TISSUES

Tissue from the intima/media layer of mammary artery (n=88) and heart (n=139) were collected from Swedish patients undergoing heart valve surgery (Paloschi, Kurtovic et al.). The intima/medial and adventitial layers of the mammary artery were separated by adventicectomy. Biopsies, heart tissue from transgenic mice and Milan rats were directly incubated with RNAlater (Ambion, Austin, TX) and homogenized with a FastPrep using Lysing Matrix D tubes (MP Biomedicals, Germany). Total RNA was isolated using Trizol (BRL-Life Technologies) and RNeasy Mini kit (Qiagen, Crawley, UK) including treatment with RNase-free DNase (Qiagen) according to manufacturer's instructions. The quality of RNA was analyzed with an Agilent 2100 bioanalyzer (Agilent, Santa Clara, CA) and quantity was measured using a NanoDrop (Thermo Scientific, Waltham, MA). The RNA from human biopsies was hybridized and scanned at the Karolinska Institute 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. The RNA from mice and rats tissues was analyzed using TaqMan

5.9 IN VITRO SIK PHOSPHORYLATION

Peptides for HDAC5 fragments (S259): LRKTA**S**EPNL, S498: LSRTQ**S**SPLP, S661:LGRTQ**S**SPAA; bold **S** indicates the candidate for the phosphorylation site of SIK1; were expressed as 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^{32}P]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.

5.10 INTRACELLULAR PH DETERMINATION

pH measurements were performed using trapped fluorescent indicator method with 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF) as the fluorescent indicator. A non-charged, membrane-permeant derivative of BCECF, acetoxymethyl ester BCECF-AM was used to load a cell sample which then diffuses through the cell

membrane and is cleaved by cell esterases to form BCECF which then fluoresces according to the intracellular pH. Cells were grown in 96-well plates, as described previously (Gomes and Soares-da-Silva 2006). Cell culture medium was aspirated, and the cell monolayers were incubated for 30 min with 10 mM BCECF-AM at 37°C in 5% CO^2-95% air atmosphere. Cells were placed in the sample compartment of a dual-scanning microplate spectrofluorometer (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 (pH_i) values by comparison with values from an intracellular calibration curve using the nigericin (10 μM) and high-K^+ method (Gomes and Soares-da-Silva 2006; Pinto, Pinho et al. 2008).

5.11 DETERMINATION OF SIK1 AND SIK2 ACTIVITY

A7R5 vascular smooth muscle cells were independently transfected with GST-SIK1-^{15}Gly or GST-SIK1-^{15}Ser isoforms (GST-SIK1 either bearing the Gly or Ser at position 15). After expression, SIK1 proteins were isolated. All samples were run on one and the same SDS-PAGE and Western Blot was performed. The membranes were probed with an antibody against phosphorylated (pT182) SIK1 residue (this phosphorylation site reflects activation of SIK1 and, hence, activity) and with a polyclonal antibody against SIK1 that recognize multiple epitopes in the protein (reflecting total amount of SIK1 on the blot). SIK1 activity was expressed as the ratio between the phosphorylated (pT182 antibody against phosphorylated SIK1) and the total amount of SIK1 expressed (SIK1 antibody), as previously described (Stenstrom, Takemori et al. 2009).
HL-1 cells that had been transformed with pEBG-SIK2 (expression vector for GST-fusion protein) (Horike, Takemori et al. 2003) were lysed with 1 ml of IP lysis buffer (Horike, Takemori et al. 2003). GST-SIK2 protein was purified with glutathione-Sepharose column (GE-Healthcare). To detect SIK2 and phospho-SIK2 (pT175), anti-SIK2 antibody and anti-phospho-SIK1 antibody (pT182) that recognize the pT175 (phosphoaminoacid that reflects activation in SIK2 protein) were used. All samples were run on one and the same SDS-PAGE and WB was performed. The membranes were probed with an antibody against phosphorylated SIK1 and with a polyclonal antibody against SIK2 that recognize multiple epitopes in the protein (reflecting total amount of SIK2 on the blot). SIK2 activity was expressed as the ratio between the phosphorylated (pT182 antibody) to the total amount of SIK2 expressed.

5.12 BLOOD PRESSURE DETERMINATION IN ANIMALS

Systolic blood pressure was measured in Milan normotensive and hypertensive rats by tail plethysmography (BP recorder, U. Basile, Varese, Italy) in conscious rats. The tail cuff method was used for measuring blood pressure in *sik2*$^{+/+}$ and *sik2*$^{-/-}$ mice. After five minutes of applying the cuff to the mice tail, blood pressure variations were monitored using a digital recorder K2000ST as indicated by the manufacturer (Muromachi Kagaku Co. Ltd., Tokyo, Japan) (Matoba, Doyama et al. 2001).

6 RESULTS AND DISCUSSION

The results of these studies revealed the importance of the SIK1 as a molecule involved in the BP regulation. SIK1 by itself or in combination with humoral factors could influence BP in different populations. Moreover, the SIK1 protein and the SIK1 network were found to be implicated in the process leading to the activation of important transcription factors such as MEF2 and NFAT and several genes that are involved in CH development. The novel finding was that salt could, via the SIK network, influence the development of CH independently of BP in different mouse strain models and *in vitro* studies salt affected expression of genes associated with CH via the SIK network. Altogether these data support the theory that salt is an important factor affecting BP and independently of BP can influence the development of CH and that SIK network could be one of the key regulating pathways in the development of these pathological conditions.

6.1 SALT-INDUCIBLE KINASE 1 INFLUENCES NA^+,K^+-ATPASE ACTIVITY IN VASCULAR SMOOTH MUSCLE CELLS AND ASSOCIATES WITH VARIATIONS IN BLOOD PRESSURE (ARTICLE I)

Essential hypertension is a multifactorial condition that remains a major worldwide problem. Salt diet and genetic preconditions are thought to be some of the factors involved in development of essential hypertension. In kidneys and in vasculature a key enzyme involved in sodium regulation is Na^+/K^+-ATPase. Recently a novel salt-inducible kinase 1 (SIK1) has been discovered that is involved in cell salt homeostasis and Na^+/K^+-ATPase activity regulation. An SNP in the coding region of human *SIK1* (rs3746951) is described where a change from amino acid C → T leads to a change in the protein product from glycine to serine. This SNP occurs at a high frequency of 0.25 in the population. The sequence where this SNP is located bears very little similarity to SIK2 and SIK3 proteins meaning that if this change affects SIK1 function it would affect SIK1 dependent systems but not systems that are dependent on SIK2 and SIK3.

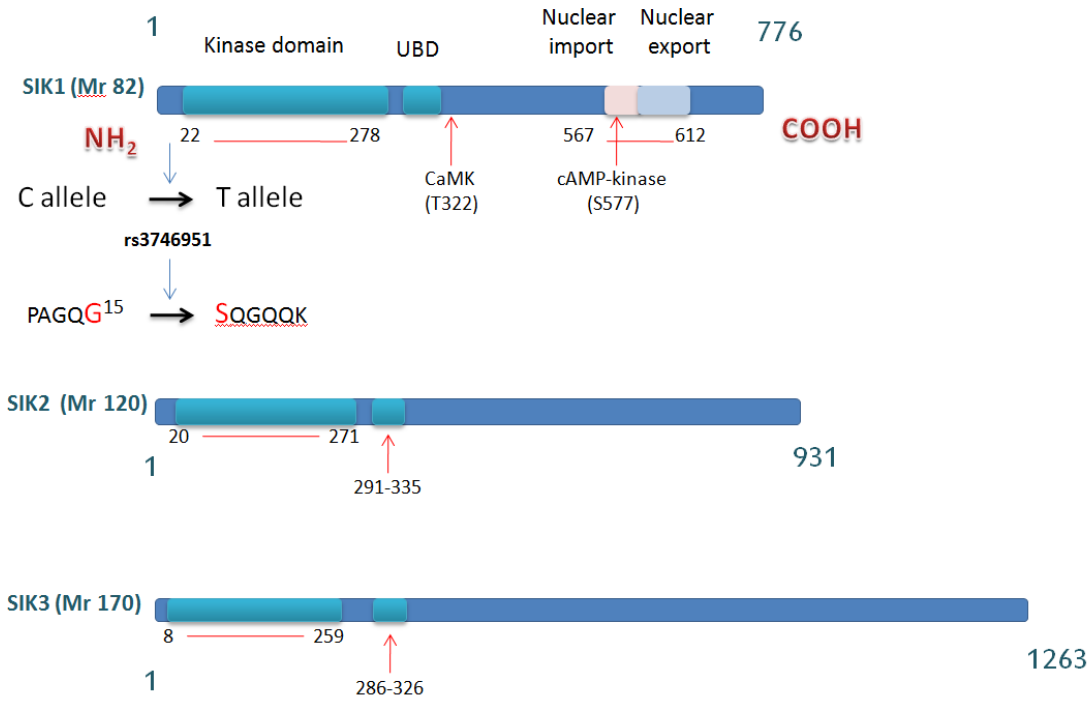

Figure 4. A schematic map of the rs3746951, the SIK isoforms and the location of the functional regions. rs3746951 is located next to the kinase domain of SIK1 protein. A substitution in human SIK1 gene, exon 3, (C → T) leads to amino acid change ^{15}Gly → Ser in the final protein product.

Therefore we hypothesized that this SNP could affect SIK1 function and SIK1 dependent pathways and possibly influence sodium homeostasis, NK function and BP variations in humans. We started by examining the effect of the rs3746951 on SIK1 activity in a cultured VSMC cell line derived from rat aortas. Transient overexpression of either the Gly^{15} or Ser^{15} variants of SIK1 lead to a significantly higher basal SIK1 activity (using GST-isolation and Western Blot) in cells expressing the Ser^{15} compared to cells expressing the Gly^{15} variant. We then proceeded to examine the effect of the Ser^{15} variant on the NK activity. Using the $^{86}Rb^{+}$transport assay we found that cells overexpressing the Ser^{15} variant had significantly increased NK activity compared to cells transiently expressing the Gly^{15} variant. Thus we demonstrated that higher SIK1 activity in VSMC leads to higher NK activity.

Following the biochemistry studies we proceeded to examine the effect of rs3746951 in different populations. Genotype-phenotype association studies between rs3746951 and BP were performed using several population-based cohorts (Stockholm study, MPP, MDC-CC and Ohasama studies). In additive models with the minor allele as coded (rs3746951 T-allele), the point estimates of the β-coefficient were negative in all study groups, and statistically significant in the largest study (MPP). Meta-analysis of the results from all four study groups was then performed, with test of effect estimate, ES of 0, resulting in z of 2.78, P value of 0.005 for SBP, and z of 3.06, P value of 0.002 for DBP, meaning that the presence of the rs3746951 is associated with both

lower SBP and DBP pressures. However, there was no significant association between the rs3746951 and hypertension. Because left ventricular mass reflects the pathophysiology of hypertension we examined association between left ventricle mass and BP in a sub set of the MPP study. The Ser^{15} variant was significantly associated with lower LV mass which confirmed the previous results of lower BP and rs3746951 association.

Our group has previously shown that SIK1 is involved in a salt-sensitive network that affects NK activity (Sjostrom, Stenstrom et al. 2007). The Gly → Ser^{15} could change the way SIK1 interacts with other proteins involved in this network or involve other unknown partner molecules that cause higher SIK1 activity. High NK activity means that more sodium is excreted from VSMC when the cells are expressing the Ser^{15} variant. This could set a lower vascular tone through lower activity of the NCX which leads to lower concentrations of calcium ions in the cell and cause cell relaxation. The precise molecular pathways and how the rs3746951 is affecting the NK are still to be elucidated however; since this SNP is adjacent to the kinase region of SIK1 one could speculate that rs3746951 influences SIK1 protein structure or/and its kinase activity which in turn could have an effect on NK activity. Alternative explanations are that higher NK activity can lead to lower BP levels by making vasculature less susceptible to OLF that are released at increased sodium levels or, that the higher activity of NK in endothelial cells may lead to hyperpolarization which decreases release of endothelin resulting in decreased BP.

Data generated from animal studies and human studies indicate that the genetic factor contributing to hypertension accounts for 30-50%. Because essential hypertension involves small effects of multiple genes and environmental factors it is not surprising that the effect of rs3746951 on hypertension was small and not significant. However, these results point to the SIK1 as a possible candidate gene that could be involved in BP regulation.

A caveat in this study was the lack of data describing levels of salt intake in all four populations. However, rs3746951 affected the SBP and DBP in both Japanese and Swedish populations, which is of importance because average levels of salt intake in Japan are higher than in Sweden (11.7 and 9 g/day respectively at the time of recruitment of participants) (Brown, Tzoulaki et al. 2009; Ogihara, Kikuchi et al. 2009). To further examine the effect of rs3746951 on BP, population-based studies with documented sodium intake / sodium excretion would be necessary. The importance of rs3746951 in the kidneys can also be examined since rs3746951 is affecting NK activity and the NK is affecting sodium re-absorption rate in the kidneys.

In summary, in this article we confirmed the importance of SIK1 for the BP regulation by both population-based studies and in cell based experiments. Rs3746951 appears to be affecting BP regulation possibly in combination with humoral factors, salt intake and lifestyle.

6.2 INCREASES IN INTRACELLULAR SODIUM ACTIVATE TRANSCRIPTION AND GENE EXPRESSION VIA THE SALT-INDUCIBLE KINASE 1 NETWORK IN AN ATRIAL MYOCYTE CELL LINE (ARTICLE II)

Cardiac hypertrophy means enlargement of the heart due to increased size of cells and is divided in two general forms: pathological hypertrophy and physiological hypertrophy. Physiological hypertrophy is a natural response to increased work-load on the heart such as heavy exercising or pregnancy and is reversible. Pathological hypertrophy is not reversible unless treated and results in abnormal mechanical stress usually caused by valve stenosis or hypertension. Molecular pathways that are initiated during pathological hypertrophy are somewhat similar to those involved in physiological hypertrophy, but do include different intracellular signaling systems. Hypertension is one of the major causes of CH, however, in certain animal models salt intake can result in hypertrophy without significant increases in BP and without involvement of the humoral factors and the RAAS system (Schmieder, Messerli et al. 1988; Frohlich, Chien et al. 1993). SIK1 has been shown to be involved in sodium regulation in cells (Sjostrom, Stenstrom et al. 2007) and its expression levels increase in adrenal medullas of rats that are fed high salt diet. SIK1 has also been coupled with transcription factors that are involved in the development of CH such as CREB regulation via the TORC-SIK1 cascade (Takemori, Kajimura et al. 2007) and class II HDAC phosphorylation which increases MEF2 activity (Berdeaux, Goebel et al. 2007). Therefore we hypothesized that salt could activate genes and transcription factors that are involved in the development of CH independently of increases in BP and that SIK1 could be a key mediator in such system.

We set out to establish whether transient sodium increases in cultured cells could affect expression levels of BNP, β-MHC and α-MHC. These genes have been coupled with the development of hypertrophy. To this end, cultured atrial cells (HL-1) were used. These cells can be serially passaged, are able to contract and share biochemical properties of the adult cardiac myocytes (Claycomb, Lanson et al. 1998). Intracellular sodium increase was achieved using sodium ionophore monensin (Efendiev, Bertorello et al. 2002). After performing either Luciferase or TaqMan assays we discovered that all of the examined genes had a time dependent increase in response to increased sodium concentrations.

Next, we proceeded to establish whether the transcription factor (TF) MEF2, which is commonly associated with the induction of hypertrophic genes, was also activated by sodium. MEF2 is a TF that is relevant for normal cardiac growth and has also been coupled with activation of hypertrophic genes. Therefore we examined the response of MEF2 to sodium. Cells transiently expressing the luciferase-tagged MEF2 vector had a significant increase in MEF2 activity in response to sodium induced both by monensin and a sodium ion channel activator veratridine.

SIK1 has been recognized as critical molecule for myocardium development (Romito, Lonardo et al. ; Ruiz, Conlon et al. 1994) and is also involved in a sodium-sensitive pathway that regulates NK activity in cells (Sjostrom, Stenstrom et al. 2007). We hypothesized that SIK1 could mediate the response of *BNP* and *SkA* hypertrophic genes to sodium. After establishing that SIK1 mRNA levels had similar increases in response to sodium as the hypertrophic genes, we used SIK1 specific siRNA to knock down SIK1 and examined BNP and SkA mRNA levels using TaqMan assay. Knocking down SIK1 led to a significant decrease of the hypertrophic genes. To further ascertain SIK1 as the mediatory molecule, we double-transfected HL-1 cells with luciferase tagged plasmids MEF2, BNP, β-MHC and with either SIK1-WT or SIK1-K56M mutant lacking the kinase activity. Cells expressing the mutant had demonstrated significantly lower luciferase activity of MEF2, BNP and β-MHC in response to monensin.

We then confirmed the association between hypertrophic genes and SIK1 by performing correlation studies in human heart biopsies (in both mammary artery tissue and heart tissue). SIK1 was found to be positively and significantly correlated with *β-MHC*, *BNP* and *SkA* genes (with exception of SkA in the mammary artery tissue). How does SIK1 mediate the sodium signals to the hypertrophic genes? Many of the intracellular pathways that lead to the development of hypertrophy in heart cells are transduced by calcium signaling (Frey and Olson 2003). Sodium influx can induce activation of the reverse mode NCX and thereby increase the concentration of calcium ions in the cell. Our group has previously demonstrated that SIK1 can be activated by influx of sodium resulting in activation of reverse mode of NCX and subsequent CaMK phosphorylation of SIK1 (Stenstrom, Takemori et al. 2009). We tested the dependence of MEF2 on calcium in HL-1 cells after transfecting the cells with luciferase tagged MEF2 plasmid. This was done in three separate experiments: by blocking NCX channels, by quenching intracellular calcium and by inhibiting CaMKII. In response to monensin, all three experiments led to a significantly lower MEF2 luciferase activity when the calcium pathway was abrogated.

The last step in elucidating the pathway from sodium influx to activation of hypertrophy genes was examining the link between SIK1 and MEF2. It has been previously demonstrated that SIK1 phosphorylates class II HDAC (Berdeaux, Goebel et al. 2007). Class II HDAC binds to MEF2 directly in the nucleus and represses its activity. Phosphorylation of HDAC leads to its dissociation from MEF2 and translocation from the nucleus. We tested whether transient increases of sodium could activate MEF2 in presence of class II HDAC specific blocker or in cells that transiently expressed HDAC lacking SIK1 phosphorylation domain. Cells that were pre-treated with the class II HDAC inhibitor or expressing the HDAC mutant had significantly lower MEF2 luciferase activity in response to monensin compared to cells without the inhibitor or expressing the wild type HDAC.

Influx of sodium induced by monensin, lead to a concomitant influx of calcium ions via the reverse mode of NCX. This activated CaMKII and lead to phosphorylation of SIK1 and subsequent phosphorylation of class II HDAC. HDAC dissociates from MEF2 which then activates genes associated with CH: *BNP*, *SkA*, *β-MHC* and *α-MHC*. As many reports indicate, transcription factors associated with CH often interact with each other to promote gene transcription. NFAT is a critical transcription factor involved in development of CH. NFAT can bind to GATA4 and activate transcription of BNP (Morimoto, Hasegawa et al. 2001). NFAT can also bind to MEF2 and enhance its activity in response to calcineurin (van Oort, van Rooij et al. 2006). Therefore we hypothesized that NFAT could act together with MEF2 in response to increased sodium concentrations. We performed transfection experiments where cells were expressing luciferase-tagged NFAT plasmid and were co-transfected with either the SIK1-WT or SIK1-K56M mutant. As expected, there was significantly lower luciferase activity of NFAT if cells were expressing the SIK1 mutant compared to cells expressing the SIK-WT. Similarly to MEF2, the NFAT luciferase activity was blocked by the class II HDAC inhibitor or by cells expressing the HDAC mutant lacking the SIK1 phosphorylation site for SIK1.

Usually α-MHC is observed to be down-regulated during the development of pathological hypertrophy (Kinugawa, Yonekura et al. 2001). However, as in the case of calcineurin-mediated hypertrophy, calcineurin inhibitor MCIP can affect molecular pathways of both physiological and pathological hypertrophies (Rothermel, McKinsey et al. 2001; Hill, Rothermel et al. 2002) suggesting that even if the two hypertrophies differ somewhat at the molecular signaling level, certain genes and pathways may be involved in both forms of hypertrophy.

It has been shown that by inhibiting MEF2, hypertrophy can be suppressed (Lu, McKinsey et al. 2000). However the suggested signaling mechanism affecting MEF2 was mediated by CaMK and calcium. Here we demonstrate that sodium could be responsible for increasing MEF2 activity. Therefore we not only confirmed previous findings that calcium is involved in the signal transduction that activates MEF2 and CH genes, but also discovered that sodium could be an upstream regulator of these genes. There have not been any reports indicating that HDACs can bind to NFAT, however, because the MEF2 and NFAT can co-activate hypertrophic genes it is plausible that SIK1 phosphorylation of HDAC lead to binding of MEF2 to NFAT or vice-versa thereby promoting gene transcription. There is also a phosphorylation consensus sequence for SIK1 on NFAT which means SIK1 could phosphorylate both HDAC and NFAT directly.

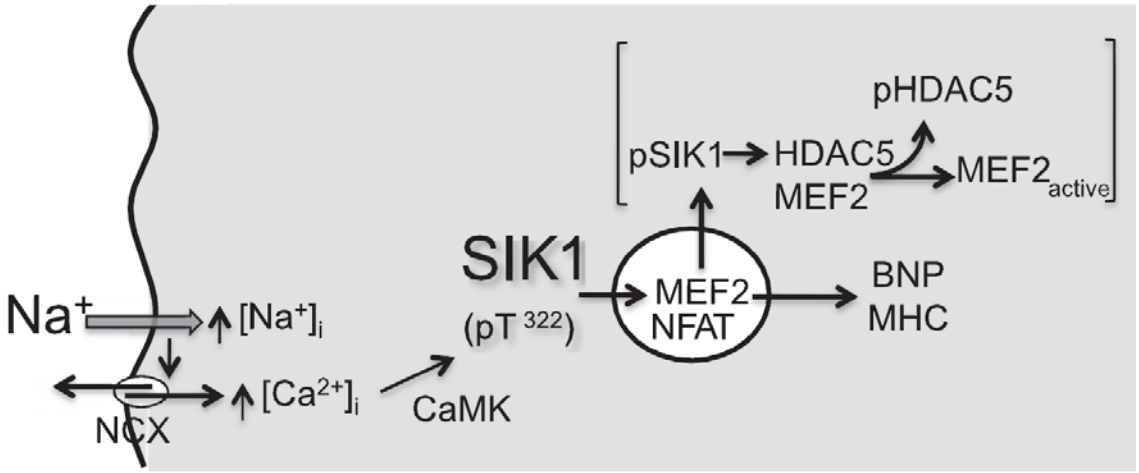

Figure 5. A schematic representation of the way that sodium might affect the hypertrophic genes. Increases in intracellular sodium lead to concomitant increases in intracellular calcium via the reverse mode of NCX. Increased calcium concentration activates CaMK which phosphorylates SIK1. The activated SIK1 phosphorylates class II HDAC which then dissociates from MEF2 and translocates from the nucleus. MEF2 activity increases and is able to activate genes that are associated with CH. Whether MEF2 somehow activates NFAT or whether SIK1 directly phosphorylates NFAT is yet unclear.

6.3 GENETIC PREDISPOSITION OR HIGH SALT INTAKE CAUSE CARDIAC HYPERTROPHY THROUGH ACTIVATION OF SIK2 INDEPENDENTLY OF BLOOD PRESSURE LEVELS (ARTICLE III)

Because we demonstrated that SIK1 could be a mediator molecule in response to sodium we hypothesized that other SIK isoforms could also be involved in regulation of CH genes and transcription factors independently of changes in arterial BP. It is also possible that other SIK isoforms are involved in cellular response to the changes in sodium concentrations. Adducin is a cytoskeletal protein that is involved in spectrin-actin binding, controls the rate of actin polymerization and is dependent on calcium (Bianchi, Ferrari et al. 2005). It consists of three subunits encoded by genes *ADD1* (α-adducin) *ADD2* (β-adducin) and *ADD3* (γ-adducin). Several association studies have linked an SNP in the *ADD1* and hypertension in different populations (Cusi, Barlassina et al. 1997). Same alteration in α-adducin gene has been implicated in development of hypertension through increased renal re-absorption of sodium (Bianchi, Tripodi et al. 1994). Since CH could also be affected by genetic predisposition we hypothesized that adducin could be a gene that affects SIK proteins and influence the development of CH. We hypothesized that adducin could be involved in CH development independently of changes in BP.

To examine the effect of adducin on CH we used a strain of Milan hypertensive rats (MHS) with a mutation in the α-adducin gene (*Add1* Phe316Tyr) (Bianchi 2005) that causes the rats to develop hypertension with age. The rats that were used were 30 days

old and were in a period before they develop hypertension (MHS-pre). These rats had, as expected, normal BP when compared to normotensive controls but had significantly higher expression of *Nppb* and *β-mhc* genes that are coupled with hypertrophy. Moreover, the expression of SIK2 was higher in the MHS-pre rats compared to normotensive control rats. These initial results suggested that CH genes were not dependent on BP and also that SIK2 could be involved in CH development. Surprisingly SIK1 levels were not increased in contrast to our previous finding that SIK1 mediated the increase in *NPPB* and *MHC* gene expression. However the system that was used in the previous study was different (cultured atrium cells) and it is possible that both SIK1 and SIK2 are relevant, depending upon the cell system. Because SIK2 levels were increased in the MHS-pre rats that have a hypertensive form of adducin we performed adducin specific knockdown in HL-1 cells using siRNA. The knockdown of α-adducin lead to a significant decrease of SIK2 levels and knockdown of γ-adducin lead to a reduction of SIK2 but not a significant one. To confirm the dependency of SIK2 on adducin we analyzed whether there were any correlations between SIK2 and adducin isoforms in biopsies from human subjects undergoing heart valve surgery. The results revealed that there was indeed a positive and significant correlation between SIK2 and γ- and α-adducins.

We further explored the dependency of the CH genes on adducin and SIK2 by analyzing correlations between the CH markers and SIK2 and adducins in the same biopsies. There were positive and significant correlations between SIK2 and the TF MEF2, β-MHC and ACTA1. In addition, γ- and α-adducins were positively correlated with MEF2, β-MHC and ACTA1. Based on these results we speculated that the presence of the hypertensive form of adducin could, via SIK2 trigger activation of MEF2 and subsequently lead to an elevation of CH-associated genes. Surprisingly SIK2 levels were not different in HL-1 cells transiently expressing either normotensive or hypertensive form of α-adducin. The SIK2 activity, whereas higher in the cells with hypertensive form of α- adducin, did not reach a statistical significance. This could mean that the experimental system (MHS rats or *in vitro* studies) play a role. We examined whether the CH markers and MEF2 expression was mediated by SIK2 in HL-1 cells expressing either the normotensive or hypertensive form of α-adducin together with either the SIK2 WT or a SIK2 mutant K49M lacking kinase activity and found that HL-1 cells expressing SIK2 WT and the hypertensive form of α-adducin had significantly increased MEF2, NPPB and β-MHC luciferase activities compared to the cells expressing normotensive variant. This effect was absent in cells expressing the SIK2 mutant which further confirmed that SIK2 mediates the effect of α-adducin on genes coupled with CH.

To further elucidate the importance of SIK2 on the CH development and its possible function as a sensor of sodium imbalances, we used transgenic mice with ablated SIK2. Four month old wild type mice that were fed high salt diet (1% saline) had a

significantly increased heart weight compared to control animals fed tap water. This increase was absent in *sik2*$^{-/-}$ mice which were fed the same salt diet.

These results demonstrate that SIK2 is one of the key molecules involved in salt-dependent CH. In our previous article we demonstrated that SIK1 was the key protein mediating the effects of increased salt on MEF2 and NFAT and on the genes associated with CH. However, these results demonstrate that in rats and mice SIK2, not SIK1 is responsible for the events that can cause CH. It is possible that SIK1 could have a compensatory effect when the SIK2 is absent and a double knockout/overexpression of SIK1 and SIK2 would be ideal to examine whether SIK1 and SIK2 contribute equally to the development of CH in different experimental models. Nevertheless it appears that SIK2 is an important mediator of sodium effect on the development of CH. These results also indicate that the presence of the hypertensive form of α-adducin *per se* represents a risk factor for the development of CH. The α-adducin but not γ-adducin appears to be a regulator of SIK2 expression and activity. The mechanism by which adducin controls SIK2 activity or expression remains to be explored.

7 SUMMARY AND CONCLUSION

Recently, salt-inducible kinase 1 and its isoforms have emerged as proteins participating in several intracellular mechanisms, involving a wide range of pathways such as cell cycle regulation, cell sodium homeostasis and steroidogenesis and adipogenesis. The projects presented in this dissertation were aimed towards exploring the involvement of the salt-inducible kinases and SIK network in the development of hypertension and hypertrophy.

The findings presented in this thesis provide further support for the theory that sustained changes in BP and subsequent mechanical load on the heart are not necessary for the development of CH. This thesis also confirms previous knowledge about the sodium effect on the BP and suggests the importance of SIK1 in the development of hypertension.

In **Article I** we demonstrated that genetic variation within SIK1 affects systolic and diastolic blood pressure in different populations, affects left ventricular mass and increases the Na^+/K^+-ATPase activity in cultured vascular smooth muscle cells. We conclude that SIK1, possibly in combination with humoral and life-style factors, is important for BP regulation and could be a potential drug target.

In **Article II** we explored the possibility of SIK1 being involved in a process that up-regulates genes and transcription factors coupled to development of hypertrophy in cultured myocytes in response to salt. We found that SIK1 mediated the effect of sodium on the gene expression and protein levels of key transcription factors and genes involved in development of CH. We conclude that it is plausible that hypertrophy can develop without sustained increases in BP in response to salt and that SIK network is a key pathway involved in this process.

In **Article III** we utilized different experimental systems to prove that salt and a mutation within cytoskeletal protein α-adducin *per se* can affect development of CH independently of high BP. Human, rat, mouse models as well as cell based experiments, confirmed our theory. We conclude that SIK2 mediates the effect of α-adducin on the transcription factor MEF2 and genes coupled with hypertrophy and that blocking SIK2 could provide novel therapeutic approach to prevent CH in salt-sensitive individuals.

8 ACKNOWLEDGEMENTS

This thesis was performed at the Center of Molecular Medicine and Membrane Signaling Networks within the Atherosclerosis Research Unit in the Department of Medicine, Karolinska Hospital. I would like to express my sincere gratitude towards my supervisors, colleagues and friends that helped me to go through these years of my thesis work.

My main supervisor, **Alejandro Bertorello**, thank you for your patience and guidance during these years. You have taught me a lot about science. You are an exceptional researcher and I wish you all the best in your continued scientific career.
My co-supervisor, **Ferdinand Van´t Hooft,** you took me under your wings and introduced me to the world of science. The only thing that could come between you and me, Japan, happened and we had to part our ways but I am very happy that you accepted me back and grateful for your advice both in private life and in science.
My mentor **Lars Ryden,** I would literally not been able to survive as a PhD student without you. Thank you for your mental support.
Peter Möller, thank you for staying a true friend of my family and for your kindness and helpful advice throughout all these years.
Per Eriksson, as usual in our department, you acted as my co-supervisor. Thank you for all your contribution to the articles and for all your help during the writing of the thesis.
Anders Hamsten, thank you for all your generosity, your help, your advice and for being an exceptional leader of our department.
Hiroshi Takemori, The silent contributor to all my work. I have never met you but none of the articles would have been possible without you.
Naoki Mochizuki, thank you for introducing me to the world of FRET and for the warm welcome to your lab and to Osaka.

Present and past members of the MSN group. **Kristina Eneling,** thank you for your cheerfulness and all the long conversations. I learned more than I ever wanted about what it is like to carry a baby. Some details were very descriptive; **Laura Brion,** thank you for all the friendly conversations and for all your help with my work. It was a pleasure working and going out with you. I hope I did not do any damage to your liver; **Paula Ciscotto,** thank you for being a good friend and for all the advice on the private life. Do not ever let this country change you; **Rodrigo Novaes Ferreira,** thank you for your happy mood and for lighting up the office with your personality; **Karin Stenström,** it was a pleasure working with you. Thank you for your co-operation and for all the advice on the office politics; **Mattias Sjöström,** Too bad I never got the

chance working with you. Thank you for the advice on the personal level; **Zongpei Chen,** thank you for all the friendly advice regarding job-searching.

The Atherosclerosis group and colleagues from CMM**. Shohreh Maleki,** thank you very much for all the help with my articles, my thesis and the questioning. You helped me to improve my writing; **Rona Strawbridge,** thank you so much for all the help with my thesis and the questioning me on my articles. Good luck with your bicycling! **Rachel Fisher,** thank you for the help with preparation of the article presentation; **Ewa Ehrenborg,** thank you for all the discussions during seminars; **Joanna Chmielewska,** congratulations to you and Kalle! Good luck with you work; **Maria Iglesias,** thank you for the help with the TaqMan; **Karin Husman,** thank you very much for all the help in the lab and for helping me to keep track on where all the things should be in the lab; **Karl Gertow,** nice to have you back. Congratulations with your marriage! **Dick Wågsäter,** thank you for teaching me immunohistochemistry and for your co-operation on my articles; **Therese Olsson,** thank you for your help in the lab and your friendly personality; **Lasse Folkersen,** thank you for all the advice during the seminars and for interesting discussions during the group meetings; **Maria Nastase Mannila,** my ”Italian wife”. Good luck with everything; **Magnus Mossfeldt,** thank you for all the help with the IT stuff; **Karin Danell-Toverud,** thank you for helping me organizing the product ordering for our group; **Angela Silveira,** thank you for all the help regarding genetics and for co-operating on my article; **Ann-Marie Björkholm,** thank you for all the help with the administrative things. I would be totally lost without you; **Hovsep Mahdessian,** thank you for making me laugh doing the baby noises and for all the friendly chats; **Petra Thulin,** thank you for the advice regarding Luciferase assay; **Fariba Foroogh,** thank you for all the help in the lab and for being such a friendly and likeable person; **Valentina Paloschi,** thank you for your cheerful personality. Do not ever change; **Louisa Cheung,** thank you for contributing to a nice atmosphere during the group seminars; **Josefin Skogsberg,** thank you for organizing all the sketches. Nobody does it better than you; **Sarah-Jayne Reilly,** good luck in San Francisco; **Emina Vorkapic,** thank you for the friendly chats. Good luck in Linköping; **Olivera Werngren,** thank you for relieving me from the isotope room duty; **Barbro Burt,** thank you for making me feel home in the lab when I first came here; **Per Sjöström,** thank you for organizing the poker club even though I have never won anything; **Hanna Björk,** thank you for the advice regarding immunohistochemistry staining; **Massimiliano Ria,** thank you for all the football discussions and fighting during our countless lunches together; **Anna Aminoff,** thank you for your endless energy defending the rights of PhD students; **Anton Ruzuvaev,** thank you for the coffee machine; **Justo Sierra Johnson,** thank you for introducing me into the Stockholm night-life. My wallet disagrees with me though; **Elvi Sandberg,** thank you for the help with the beta counter; **Siw Frebelius**, thank you for all the help with the

logistics; **Berit Björkman,** thank you for the kind words and support during writing of the thesis; **Alexandra Bäcklund, Alexander Kovacs, Maria Kolak, Joëlle Magné, Maria Gonzalez, Maria Sabater Lleal, Peri Noori, Sanela Kurtovic, Anders Mälarstig, Tianling Wei, Jacob Odeberg, Bengt Sennblad, Johan Björkegren, Kerstin Lundell, Ann Samnegård, Birgitta Söderholm, Malin Larsson, John Öhrvik, Vincent Fontaine, Monzur Kazi, Fei Chen,** and all other, thank you all for contributing to a pleasant working atmosphere.

Sergey, you have always been there for me my friend. No matter if it concerned work or my private life and no matter if you were in Denmark or in Sweden, I could always rely on you whenever I had trouble. I always look forward to the brief periods of time you are here. Beware though: I am coming to Copenhagen soon.
Katja, not sure if I would manage to survive in the lab without you. Thank you for all the personal talks and for being a true friend during all this time. I look forward to traveling with you again.

The Uppsala Gang, thank you for remaining my friends during all these years. **Chia,** our friendship proves that no matter where on earth you live true friends will always remain friends; **Salah,** I am surprised I am still alive considering the liver damage our parties must have caused. Wish you all the best in Stanford; **Safah,** I wish all the best to you and to your beautiful wife Parastoo; **Diana,** thanks for keeping Salah on the leash and for all the fun memories. That tequila party was legendary.

Olga, thank you for the help with the questioning and for being a good friend. Look forward to another sambuka evening. **Mike,** face it man, Arsenal will never win the PL again. See you in the nearest pub when the season kicks off; **Sun,** thanks for the friendly conversations during the long and boring winter evenings; **Marcelo,** good luck in Mexico man. Looking forward seeing your wife soon. I hope everything will turn out great for you two in Sweden.

Christian, you have to make up your mind where you want to live my friend. I know though, that no matter where you are we will always remain great friends. Thanks for your friendship and support and for everything you have done for me; **Markus,** you have to stop traveling so much. I miss you and our evenings with Chris. Wish all the best to you and Natalia; **Manne,** do not get too addicted googling naked models; **Micke,** I hope you can surpass Christian some day in you running ability; **Johnny,** thank you for being a great friend during all these years. I have lost track of our whisky evenings; **Robban,** I wish you all the best in your adventure in Japan; **Anna, Natalia, Nina,** Markus got three girlfriends instead of one. Not sure if I should be jealous or pity him.

Mahdi, going to the gym will never be the same without you. But please, no more tattoos; **Chiara,** thank you for taking care of Chia. You two are the perfect match and I wish you all the best; **Melissa,** take care of Rodrigo. I wish you all the best in your Swedish adventure; **Choon,** thanks for cheering me up on Facebook. Good luck in Stockholm; **Pedro,** Barney my man, you are awesome. I would never have met my lovely Vesela if it were not for you. Thank you for everything, but mostly for becoming a great friend in such a short time; **Dilian,** thank you for all the personal advice and for taking care of Vesela.

Vesela, my love, I did not know what my life was missing until I met you. What I want to tell you and what I feel for you is not something that can be put down on paper. A great adventure awaits us and I have never felt this excited in my life.

My Mother, you always believed in me and supported me, no matter what I did. Thank you for your endless love. I love you very much

9 REFERENCES

(1988). "Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. Intersalt Cooperative Research Group." BMJ **297**(6644): 319-28.

(1998). "Excerpts from the United States Renal Data System 1998 Annual Data Report." Am J Kidney Dis **32**(2 Suppl 1): S1-162.

(2003). "2003 European Society of Hypertension-European Society of Cardiology guidelines for the management of arterial hypertension." J Hypertens **21**(6): 1011-53.

(2007). "Hypertension: uncontrolled and conquering the world." Lancet **370**(9587): 539.

Adams, M. D., S. E. Celniker, et al. (2000). "The genome sequence of Drosophila melanogaster." Science **287**(5461): 2185-95.

Akazawa, H. and I. Komuro (2003). "Roles of cardiac transcription factors in cardiac hypertrophy." Circ Res **92**(10): 1079-88.

Arnett, D. K., Y. Hong, et al. (2001). "Sibling correlation of left ventricular mass and geometry in hypertensive African Americans and whites: the HyperGEN study. Hypertension Genetic Epidemiology Network." Am J Hypertens **14**(12): 1226-30.

Barroso, I., M. Gurnell, et al. (1999). "Dominant negative mutations in human PPARgamma associated with severe insulin resistance, diabetes mellitus and hypertension." Nature **402**(6764): 880-3.

Beilin, L. J., F. S. Goldby, et al. (1977). "High arterial pressure versus humoral factors in the pathogenesis of the vascular lesions of malignant hypertension." Clin Sci Mol Med **52**(2): 111-7.

Beilin, L. J., I. B. Puddey, et al. (1996). "Alcohol and hypertension--kill or cure?" J Hum Hypertens **10 Suppl 2**: S1-5.

Berdeaux, R., N. Goebel, et al. (2007). "SIK1 is a class II HDAC kinase that promotes survival of skeletal myocytes." Nat Med **13**(5): 597-603.

Bertorello, A. M. and J. K. Zhu (2009). "SIK1/SOS2 networks: decoding sodium signals via calcium-responsive protein kinase pathways." Pflugers Arch **458**(3): 613-9.

Bianchi, G. (2005). "Genetic variations of tubular sodium reabsorption leading to "primary" hypertension: from gene polymorphism to clinical symptoms." Am J Physiol Regul Integr Comp Physiol **289**(6): R1536-49.

Bianchi, G., P. Ferrari, et al. (2005). "Adducin polymorphism: detection and impact on hypertension and related disorders." Hypertension **45**(3): 331-40.

Bianchi, G., G. Tripodi, et al. (1994). "Two point mutations within the adducin genes are involved in blood pressure variation." Proc Natl Acad Sci U S A **91**(9): 3999-4003.

Bidani, A. K. and K. A. Griffin (2002). "Long-term renal consequences of hypertension for normal and diseased kidneys." Curr Opin Nephrol Hypertens **11**(1): 73-80.

Brown, I. J., I. Tzoulaki, et al. (2009). "Salt intakes around the world: implications for public health." Int J Epidemiol **38**(3): 791-813.

Carretero, O. A. and S. Oparil (2000). "Essential hypertension. Part I: definition and etiology." Circulation **101**(3): 329-35.

Cheng, J., M. Uchida, et al. "Role of salt-induced kinase 1 in androgen neuroprotection against cerebral ischemia." J Cereb Blood Flow Metab **31**(1): 339-50.

Chobanian, A. V., G. L. Bakris, et al. (2003). "The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report." JAMA **289**(19): 2560-72.

Claycomb, W. C., N. A. Lanson, Jr., et al. (1998). "HL-1 cells: a cardiac muscle cell line that contracts and retains phenotypic characteristics of the adult cardiomyocyte." Proc Natl Acad Sci U S A **95**(6): 2979-84.

Cook, N. R., J. A. Cutler, et al. (2007). "Long term effects of dietary sodium reduction on cardiovascular disease outcomes: observational follow-up of the trials of hypertension prevention (TOHP)." BMJ **334**(7599): 885-8.

Cooper, G. t., R. L. Kent, et al. (1985). "Hemodynamic versus adrenergic control of cat right ventricular hypertrophy." J Clin Invest **75**(5): 1403-14.

Cowley, A. W., Jr. (2006). "The genetic dissection of essential hypertension." Nat Rev Genet **7**(11): 829-40.

Curtis, J. J., R. G. Luke, et al. (1983). "Remission of essential hypertension after renal transplantation." N Engl J Med **309**(17): 1009-15.

Cusi, D., C. Barlassina, et al. (1997). "Polymorphisms of alpha-adducin and salt sensitivity in patients with essential hypertension." Lancet **349**(9062): 1353-7.

Devereux, R. B., G. de Simone, et al. (1993). "Left ventricular hypertrophy associated with hypertension and its relevance as a risk factor for complications." J Cardiovasc Pharmacol **21 Suppl 2**: S38-44.

Dong, X. H., Y. Komiyama, et al. (2004). "Nanomolar level of ouabain increases intracellular calcium to produce nitric oxide in rat aortic endothelial cells." Clin Exp Pharmacol Physiol **31**(5-6): 276-83.

Efendiev, R., A. M. Bertorello, et al. (2002). "Agonist-dependent regulation of renal Na+,K+-ATPase activity is modulated by intracellular sodium concentration." J Biol Chem **277**(13): 11489-96.

Efendiev, R., R. T. Krmar, et al. (2004). "Hypertension-linked mutation in the adducin alpha-subunit leads to higher AP2-mu2 phosphorylation and impaired Na+,K+-

ATPase trafficking in response to GPCR signals and intracellular sodium." Circ Res **95**(11): 1100-8.

Ehret, G. B., P. B. Munroe, et al. "Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk." Nature **478**(7367): 103-9.

Fogo, A. B. (2000). "The role of angiotensin II and plasminogen activator inhibitor-1 in progressive glomerulosclerosis." Am J Kidney Dis **35**(2): 179-88.

Franco, O. H., A. Peeters, et al. (2005). "Blood pressure in adulthood and life expectancy with cardiovascular disease in men and women: life course analysis." Hypertension **46**(2): 280-6.

Frey, N. and E. N. Olson (2003). "Cardiac hypertrophy: the good, the bad, and the ugly." Annu Rev Physiol **65**: 45-79.

Frohlich, E. D., Y. Chien, et al. (1993). "Relationship between dietary sodium intake, hemodynamics, and cardiac mass in SHR and WKY rats." Am J Physiol **264**(1 Pt 2): R30-4.

Galderisi, M., A. Celentano, et al. (1993). "Parental left ventricular hypertrophy predicts Doppler-derived diastolic filling in young offspring of hypertensive subjects." J Hypertens Suppl **11**(5): S76-7.

Gallo, E. F. and C. Iadecola "Balancing life and death in the ischemic brain: SIK and TORC weigh in." Neuron **69**(1): 3-6.

Gomes, P. and P. Soares-da-Silva (2006). "Upregulation of apical NHE3 in renal OK cells overexpressing the rodent alpha(1)-subunit of the Na(+) pump." Am J Physiol Regul Integr Comp Physiol **290**(4): R1142-50.

Graudal, N. A., T. Hubeck-Graudal, et al. "Effects of low-sodium diet vs. high-sodium diet on blood pressure, renin, aldosterone, catecholamines, cholesterol, and triglyceride (Cochrane Review)." Am J Hypertens **25**(1): 1-15.

Grisk, O. and R. Rettig (2001). "Renal transplantation studies in genetic hypertension." News Physiol Sci **16**: 262-5.

Guyton, A. C. (1991). "Blood pressure control--special role of the kidneys and body fluids." Science **252**(5014): 1813-6.

Hall, J. E. (2003). "The kidney, hypertension, and obesity." Hypertension **41**(3 Pt 2): 625-33.

Hall, J. E., A. C. Guyton, et al. (1996). "Pressure-volume regulation in hypertension." Kidney Int Suppl **55**: S35-41.

Heineke, J. and J. D. Molkentin (2006). "Regulation of cardiac hypertrophy by intracellular signalling pathways." Nat Rev Mol Cell Biol **7**(8): 589-600.

Hill, J. A., B. Rothermel, et al. (2002). "Targeted inhibition of calcineurin in pressure-overload cardiac hypertrophy. Preservation of systolic function." J Biol Chem **277**(12): 10251-5.

Horike, N., H. Takemori, et al. (2003). "Adipose-specific expression, phosphorylation of Ser794 in insulin receptor substrate-1, and activation in diabetic animals of salt-inducible kinase-2." J Biol Chem **278**(20): 18440-7.

Hsu, C. Y., C. E. McCulloch, et al. (2005). "Elevated blood pressure and risk of end-stage renal disease in subjects without baseline kidney disease." Arch Intern Med **165**(8): 923-8.

Hsueh, W. C., B. D. Mitchell, et al. (2000). "QTL influencing blood pressure maps to the region of PPH1 on chromosome 2q31-34 in Old Order Amish." Circulation **101**(24): 2810-6.

Huggett, R. J., J. Burns, et al. (2004). "Sympathetic neural activation in nondiabetic metabolic syndrome and its further augmentation by hypertension." Hypertension **44**(6): 847-52.

Jefcoate, C. R., J. Lee, et al. "cAMP stimulation of StAR expression and cholesterol metabolism is modulated by co-expression of labile suppressors of transcription and mRNA turnover." Mol Cell Endocrinol **336**(1-2): 53-62.

Johnson, R. J., K. L. Gordon, et al. (1999). "Renal injury and salt-sensitive hypertension after exposure to catecholamines." Hypertension **34**(1): 151-9.

Katagiri, H., T. Yamada, et al. (2007). "Adiposity and cardiovascular disorders: disturbance of the regulatory system consisting of humoral and neuronal signals." Circ Res **101**(1): 27-39.

Katoh, Y., H. Takemori, et al. (2004). "Salt-inducible kinase (SIK) isoforms: their involvement in steroidogenesis and adipogenesis." Mol Cell Endocrinol **217**(1-2): 109-12.

Kawano, Y. "Physio-pathological effects of alcohol on the cardiovascular system: its role in hypertension and cardiovascular disease." Hypertens Res **33**(3): 181-91.

Kimes, B. W. and B. L. Brandt (1976). "Characterization of two putative smooth muscle cell lines from rat thoracic aorta." Exp Cell Res **98**(2): 349-66.

Kinugawa, K., K. Yonekura, et al. (2001). "Regulation of thyroid hormone receptor isoforms in physiological and pathological cardiac hypertrophy." Circ Res **89**(7): 591-8.

Kira, Y., P. J. Kochel, et al. (1984). "Aortic perfusion pressure as a determinant of cardiac protein synthesis." Am J Physiol **246**(3 Pt 1): C247-58.

Kira, Y., T. Nakaoka, et al. (1994). "Effect of long-term cyclic mechanical load on protein synthesis and morphological changes in cultured myocardial cells from neonatal rat." Cardiovasc Drugs Ther **8**(2): 251-62.

Klag, M. J., P. K. Whelton, et al. (1996). "Blood pressure and end-stage renal disease in men." N Engl J Med **334**(1): 13-8.

Klatsky, A. L. (1996). "Alcohol and hypertension." Clin Chim Acta **246**(1-2): 91-105.

Komuro, I., T. Kaida, et al. (1990). "Stretching cardiac myocytes stimulates protooncogene expression." J Biol Chem **265**(7): 3595-8.

Lee, D. H., M. H. Ha, et al. (2001). "Effects of smoking cessation on changes in blood pressure and incidence of hypertension: a 4-year follow-up study." Hypertension **37**(2): 194-8.

Levy, D., K. M. Anderson, et al. (1988). "Echocardiographically detected left ventricular hypertrophy: prevalence and risk factors. The Framingham Heart Study." Ann Intern Med **108**(1): 7-13.

Levy, D., A. L. DeStefano, et al. (2000). "Evidence for a gene influencing blood pressure on chromosome 17. Genome scan linkage results for longitudinal blood pressure phenotypes in subjects from the framingham heart study." Hypertension **36**(4): 477-83.

Lewington, S., R. Clarke, et al. (2002). "Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies." Lancet **360**(9349): 1903-13.

Lifton, R. P., A. G. Gharavi, et al. (2001). "Molecular mechanisms of human hypertension." Cell **104**(4): 545-56.

Lin, X., H. Takemori, et al. (2001). "Salt-inducible kinase is involved in the ACTH/cAMP-dependent protein kinase signaling in Y1 mouse adrenocortical tumor cells." Mol Endocrinol **15**(8): 1264-76.

Lovallo, W. R. and W. Gerin (2003). "Psychophysiological reactivity: mechanisms and pathways to cardiovascular disease." Psychosom Med **65**(1): 36-45.

Lu, J., T. A. McKinsey, et al. (2000). "Regulation of skeletal myogenesis by association of the MEF2 transcription factor with class II histone deacetylases." Mol Cell **6**(2): 233-44.

Manunta, P., M. Burnier, et al. (1999). "Adducin polymorphism affects renal proximal tubule reabsorption in hypertension." Hypertension **33**(2): 694-7.

Marin, R., M. Gorostidi, et al. (2005). "Systemic and glomerular hypertension and progression of chronic renal disease: the dilemma of nephrosclerosis." Kidney Int Suppl(99): S52-6.

Matoba, N., N. Doyama, et al. (2001). "Design and production of genetically modified soybean protein with anti-hypertensive activity by incorporating potent analogue of ovokinin(2-7)." FEBS Lett **497**(1): 50-4.

McMullen, J. R., T. Shioi, et al. (2003). "Phosphoinositide 3-kinase(p110alpha) plays a critical role for the induction of physiological, but not pathological, cardiac hypertrophy." Proc Natl Acad Sci U S A **100**(21): 12355-60.

Minami, J., T. Ishimitsu, et al. (1999). "Effects of smoking cessation on blood pressure and heart rate variability in habitual smokers." Hypertension **33**(1 Pt 2): 586-90.

Molkentin, J. D., J. R. Lu, et al. (1998). "A calcineurin-dependent transcriptional pathway for cardiac hypertrophy." Cell **93**(2): 215-28.

Morimoto, T., K. Hasegawa, et al. (2001). "Calcineurin-GATA4 pathway is involved in beta-adrenergic agonist-responsive endothelin-1 transcription in cardiac myocytes." J Biol Chem **276**(37): 34983-9.

Morin, S., F. Charron, et al. (2000). "GATA-dependent recruitment of MEF2 proteins to target promoters." EMBO J **19**(9): 2046-55.

Mullins, L. J., M. A. Bailey, et al. (2006). "Hypertension, kidney, and transgenics: a fresh perspective." Physiol Rev **86**(2): 709-46.

Neuringer, J. R. and B. M. Brenner (1993). "Hemodynamic theory of progressive renal disease: a 10-year update in brief review." Am J Kidney Dis **22**(1): 98-104.

Ogihara, T., K. Kikuchi, et al. (2009). "The Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2009)." Hypertens Res **32**(1): 3-107.

Oi, S., T. Haneda, et al. (1999). "Lovastatin prevents angiotensin II-induced cardiac hypertrophy in cultured neonatal rat heart cells." Eur J Pharmacol **376**(1-2): 139-48.

Okamoto, M., H. Takemori, et al. (2004). "Salt-inducible kinase in steroidogenesis and adipogenesis." Trends Endocrinol Metab **15**(1): 21-6.

Okubo, Y., Y. Suwazono, et al. (2004). "An association between smoking habits and blood pressure in normotensive Japanese men: a 5-year follow-up study." Drug Alcohol Depend **73**(2): 167-74.

Padilha, A. S., L. V. Rossoni, et al. (2004). "Ouabain at nanomolar concentration promotes synthesis and release of angiotensin II from the endothelium of the tail vascular bed of spontaneously hypertensive rats." J Cardiovasc Pharmacol **44**(3): 372-80.

Palmer, B. M. (2005). "Thick filament proteins and performance in human heart failure." Heart Fail Rev **10**(3): 187-97.

Paloschi, V., S. Kurtovic, et al. "Impaired splicing of fibronectin is associated with thoracic aortic aneurysm formation in patients with bicuspid aortic valve." Arterioscler Thromb Vasc Biol **31**(3): 691-7.

Paulis, L. and T. Unger "Novel therapeutic targets for hypertension." Nat Rev Cardiol **7**(8): 431-41.

Pedemonte, C. H., T. A. Pressley, et al. (1997). "Stimulation of protein kinase C rapidly reduces intracellular Na+ concentration via activation of the Na+ pump in OK cells." Mol Pharmacol **52**(1): 88-97.

Pinto, V., M. J. Pinho, et al. (2008). "Oxidative stress and the genomic regulation of aldosterone-stimulated NHE1 activity in SHR renal proximal tubular cells." Mol Cell Biochem **310**(1-2): 191-201.

Popov, S., K. Venetsanou, et al. "Increases in intracellular sodium activate transcription and gene expression via the salt-inducible kinase 1 network in an atrial myocyte cell line." Am J Physiol Heart Circ Physiol.

Potthoff, M. J. and E. N. Olson (2007). "MEF2: a central regulator of diverse developmental programs." Development **134**(23): 4131-40.

Romito, A., E. Lonardo, et al. "Lack of sik1 in mouse embryonic stem cells impairs cardiomyogenesis by down-regulating the cyclin-dependent kinase inhibitor p57kip2." PLoS One **5**(2): e9029.

Rothermel, B. A., T. A. McKinsey, et al. (2001). "Myocyte-enriched calcineurin-interacting protein, MCIP1, inhibits cardiac hypertrophy in vivo." Proc Natl Acad Sci U S A **98**(6): 3328-33.

Ruiz, J. C., F. L. Conlon, et al. (1994). "Identification of novel protein kinases expressed in the myocardium of the developing mouse heart." Mech Dev **48**(3): 153-64.

Ruwhof, C. and A. van der Laarse (2000). "Mechanical stress-induced cardiac hypertrophy: mechanisms and signal transduction pathways." Cardiovasc Res **47**(1): 23-37.

Sadoshima, J., L. Jahn, et al. (1992). "Molecular characterization of the stretch-induced adaptation of cultured cardiac cells. An in vitro model of load-induced cardiac hypertrophy." J Biol Chem **267**(15): 10551-60.

Sasaki, T., H. Takemori, et al. "SIK2 is a key regulator for neuronal survival after ischemia via TORC1-CREB." Neuron **69**(1): 106-19.

Scherrer, U., D. Randin, et al. (1994). "Body fat and sympathetic nerve activity in healthy subjects." Circulation **89**(6): 2634-40.

Schmieder, R. E., F. H. Messerli, et al. (1988). "Dietary salt intake. A determinant of cardiac involvement in essential hypertension." Circulation **78**(4): 951-6.

Shin, H. H., J. Y. Seoh, et al. (1999). "Requirement of MEF2D in the induced differentiation of HL60 promyeloid cells." Mol Immunol **36**(18): 1209-14.

Sjostrom, M., K. Stenstrom, et al. (2007). "SIK1 is part of a cell sodium-sensing network that regulates active sodium transport through a calcium-dependent process." Proc Natl Acad Sci U S A **104**(43): 16922-7.

Stamler, J., G. Rose, et al. (1991). "Findings of the International Cooperative INTERSALT Study." Hypertension **17**(1 Suppl): I9-15.

Stenstrom, K., H. Takemori, et al. (2009). "Blocking the salt-inducible kinase 1 network prevents the increases in cell sodium transport caused by a hypertension-linked mutation in human alpha-adducin." J Hypertens **27**(12): 2452-7.

Taal, M. W. and B. M. Brenner (2001). "Evolving strategies for renoprotection: non-diabetic chronic renal disease." Curr Opin Nephrol Hypertens **10**(4): 523-31.

Takemori, H., J. Kajimura, et al. (2007). "TORC-SIK cascade regulates CREB activity through the basic leucine zipper domain." FEBS J **274**(13): 3202-9.

Takemori, H., Y. Katoh Hashimoto, et al. (2009). "Inactivation of HDAC5 by SIK1 in AICAR-treated C2C12 myoblasts." Endocr J **56**(1): 121-30.

Tilg, H. and A. R. Moschen (2006). "Adipocytokines: mediators linking adipose tissue, inflammation and immunity." Nat Rev Immunol **6**(10): 772-83.

Tozawa, M., K. Iseki, et al. (2003). "Blood pressure predicts risk of developing end-stage renal disease in men and women." Hypertension **41**(6): 1341-5.

Unger, T. (2002). "The role of the renin-angiotensin system in the development of cardiovascular disease." Am J Cardiol **89**(2A): 3A-9A; discussion 10A.

van Oort, R. J., E. van Rooij, et al. (2006). "MEF2 activates a genetic program promoting chamber dilation and contractile dysfunction in calcineurin-induced heart failure." Circulation **114**(4): 298-308.

van Rooij, E., P. A. Doevendans, et al. (2002). "Requirement of nuclear factor of activated T-cells in calcineurin-mediated cardiomyocyte hypertrophy." J Biol Chem **277**(50): 48617-26.

Weir, M. R. and V. J. Dzau (1999). "The renin-angiotensin-aldosterone system: a specific target for hypertension management." Am J Hypertens **12**(12 Pt 3): 205S-213S.

Wilkins, B. J., Y. S. Dai, et al. (2004). "Calcineurin/NFAT coupling participates in pathological, but not physiological, cardiac hypertrophy." Circ Res **94**(1): 110-8.

Xia, Y., Z. Zhang, et al. (2000). "The new serine-threonine kinase, Qik, is a target of the Qin oncogene." Biochem Biophys Res Commun **276**(2): 564-70.

Yamori, Y., Y. Nara, et al. (1990). "International cooperative study on the relationship between dietary factors and blood pressure: a report from the Cardiovascular Diseases and Alimentary Comparison (CARDIAC) Study." J Cardiovasc Pharmacol **16 Suppl 8**: S43-7.