Author's personal copy

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

Journal of Molecular and Cellular Cardiology 44 (2008) 228 – 237

Journal of Molecular and Cellular Cardiology

www.elsevier.com/locate/yjmcc

Review article

Molecular biology of the myocardial Na^+/H^+ exchanger

Larry Fliegel *

Department of Biochemistry, University of Alberta, Edmonton, AB T6G 2H7, Canada

Received 22 August 2007; received in revised form 23 November 2007; accepted 26 November 2007
Available online 14 January 2008

Abstract

The mammalian Na^+/H^+ exchanger is a pH regulatory membrane protein that uses the sodium gradient to translocate one intracellular proton in exchange for one extracellular sodium. There are nine isoforms of the protein with varying tissue and cellular distribution, some isoforms are predominantly intracellular. In the myocardium, the Na^+/H^+ exchanger type 1 isoform (NHE1) is the only plasma membrane isoform present in significant quantities. It plays an important role during ischemia/reperfusion damage to the myocardium and has recently been implicated in myocardial hypertrophy. The NHE1 gene is made from 12 exons and a differentially spliced version mediates Na^+/Li^+ exchange. The NHE1 promoter is regulated by several transcription factors. In the myocardium, transcription factors both proximal and distal to the start site affect expression, including AP-2 and a thyroid responsive element. Recently, reactive oxygen species have also been shown to be important regulators of the NHE1 promoter. Structural and functional analysis of the NHE1 protein has shown that transmembrane segments IV, VII and IX are important in ion transport and susceptibility to pharmacological inhibition. NHE1 protein and mRNA levels are elevated by cardiac ischemia/reperfusion, hypertrophy and acidosis. Understanding the mechanism by which NHE1 mediates transport and its regulation of expression will give novel insights into its contributions in cardiovascular disease.
© 2007 Elsevier Inc. All rights reserved.

Keywords: Cation binding; Gene splicing; Ischemia/reperfusion; pH regulation; Promoter; Sodium–hydrogen exchange; Sodium–lithium exchange; Tissue distribution

Contents

1. Introduction . . . 228
2. Cloning and distribution . . . 229
3. Mechanism of Na^+/H^+ exchange activity and inhibition . . . 230
4. NHE1 expression in the myocardium . . . 232
5. The NHE1 gene . . . 232
6. Conclusions . . . 233
Acknowledgments . . . 233
References . . . 234

1. Introduction

Na^+/H^+ exchangers (NHE) are universally distributed proteins that function in virtually all known cell types. In higher eukaryotes, this family of proteins functions to regulate intracellular pH. Through a product of metabolism, intracellular pH acidifies the cell cytosol and thus the plasma membrane form of this protein removes a single intracellular proton in exchange for one extracellular sodium to rectify this acidification [1]. In the myocardium, the maintenance of intracellular pH is critical to sustain contractility and to prevent damage. Na^+/H^+

* Tel.: +1 780 492 1848; fax: +1 780 492 0886.
E-mail address: lfliegel@ualberta.ca.

0022-2828/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.yjmcc.2007.11.016

Author's personal copy

exchange accounts for a large fraction of the pH regulatory activity that occurs across the plasma membrane. Bicarbonate-dependent transport also functions to regulate pH in the myocardium but may account for less than 50% of proton extrusion, varying in contribution with the species and conditions of measurement used [2–4]. The mammalian Na^+/H^+ exchanger is activated by decreases in intracellular pH making it more likely to be responsive to the increasing proton load that occurs during acute acid challenge [5]. The Cl^-/HCO_3 (anion) exchanger and Cl^-/OH^- exchangers tend to be less active with decreasing intracellular pH and tend to import acid suggesting they play a small role in regulation of excess intracellular protons [6]. Fig. 1 illustrates the effect of internal pH on activity of the Na^+/H^+ exchanger and the anion exchanger. Activity of the Na^+/H^+ exchanger is increased with acidosis and decreased at alkaline pH, while the reverse is true for the anion exchanger. Overall, it is clear that the Na^+/H^+ exchanger plays a critical role in dealing with intracellular acidosis, while this type of bicarbonate-dependent transporter is more important during alkalosis.

The purpose of this review is to summarize advances in the area of the biochemistry and molecular biology of the mammalian Na^+/H^+ exchanger. The emphasis is on the Na^+/H^+ exchanger in the myocardium. A number of basic advances in the understanding of the structure and function of the protein are also reviewed, in particular the mechanisms by which inhibitors affect NHE and how the protein itself functions. Inhibition of NHE in the myocardium has been shown to be beneficial during ischemia/reperfusion damage in a large number of animal studies (reviewed in [7]). In addition, beneficial effects of NHE inhibitors have been demonstrated in a variety of other systems including, in the central nervous system, in cardiac hypertrophy and in resuscitation treatment from cardiac arrest [8–10].

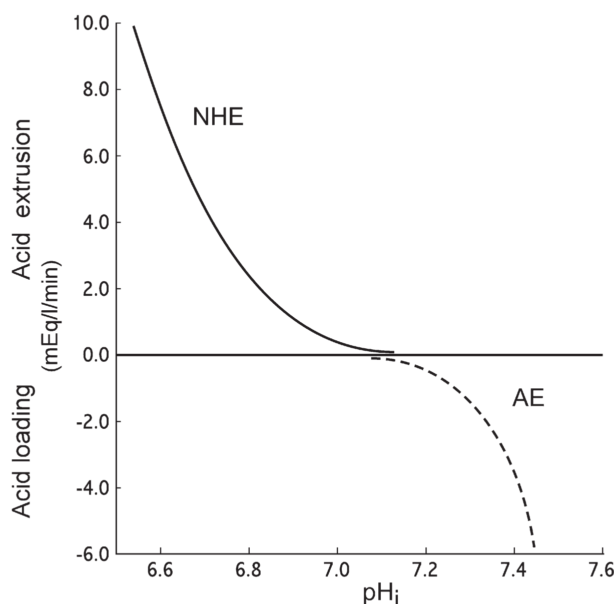

Fig. 1. Effect of changes in intracellular pH on activity of the Na^+/H^+ exchanger (NHE) and the anion exchanger (AE). Acid extrusion or acid loading are indicated. Solid line, NHE activity; dashed line, AE activity. Adapted with kind permission from Springer Science and Business Media [131].

Despite a lack of comparable success in clinical use of these inhibitors, the potential and importance of NHE inhibition during heart disease remains an outstanding incentive for the study of NHE and for understanding its mode of transport [11,12].

2. Cloning and distribution

The initial cloning of the Na^+/H^+ exchanger was accomplished by Sardet and colleagues [13]. The first isoform discovered was subsequently named NHE1 for Na^+/H^+ exchanger type 1. The human cDNA was predicted to code for a protein with two domains, a more hydrophobic membrane domain of approximately 500 amino acids and a hydrophilic carboxyl-terminal cytosolic domain of 315 amino acids. The membrane domain was predicted to contain 12 transmembrane segments. The NHE1 isoform was later cloned and sequenced from a variety of species and was shown to be highly conserved, especially in the membrane domain. The carboxyl-terminal cytosolic domain was less conserved [14]. cDNA for the NHE1 isoform was cloned from the rabbit and human myocardium and was shown to be conserved, relative to that of other tissues [15,16]. The 5-kb mRNA for NHE1 was present in whole hearts and in isolated cardiomyocytes [15,16].

NHE1 is found ubiquitously and is sometimes referred to as the "housekeeping" isoform. Subsequent to the discovery of NHE1, eight other isoforms of mammalian Na^+/H^+ exchangers have been discovered referred to as NHE1–NHE9. These are the product of different genes. NHE2–NHE5 have plasma membrane localizations, though NHE3 also has an intracellular localization that can vary in amount relative to the plasma membrane [17]. NHE2 and NHE3 are highly expressed in kidney and intestine and are predominantly located in the apical membrane of epithelia [18,19]. NHE4 is expressed in the brain, kidney, uterus and skeletal muscle and is most abundant in the stomach. Small amounts of NHE5 are expressed in non-epithelial tissues such as spleen, testis and skeletal muscle however it is mainly present in the brain [20,21]. The distribution of these isoforms in the myocardium is only partially characterized as part of other studies. NHE2 was only very faintly detected in Northern blots in the rat myocardium and Northern blot analysis and reverse transcriptase PCR detected no, or only faint, traces of NHE3 in the human and rat heart [18,22–24]. NHE4 and NHE5 were not detected in the rat myocardium by Northern blot analysis though a small amount of NHE4 antibody cross-reactive material was detected in the myocardium in one study [18,20,25].

NHE6–NHE9 are present in intracellular membranes. These include Golgi and post-Golgi compartments. They function in regulation of organelle pH and cation concentrations [26]. NHE6 to NHE9 are reported to be ubiquitous [26,27]. An initial report suggested that NHE6 was present in mitochondria however a later report demonstrated that NHE6 is not present in mitochondria and is found in recycling endosomes of cell [28 29]. NHE7 is predominantly in the trans-Golgi network and mediates influx of K^+ or Na^+ in exchange for a H^+ [30]. NHE8 is in the mid- to trans-Golgi and NHE9 is in late recycling

Author's personal copy

endosomes [26]. Specific expression of these isoforms and the regulation of their expression have not been well studied, particularly in the myocardium.

The basic structure of the Na^+/H^+ exchanger family has been most extensively studied in the NHE1 isoform. Five hundred residues make up the membrane domain that catalyzes cation transport. The balance of the protein, amino acids 501–815 mediate regulation of the protein by phosphorylation and the binding of a variety of other regulatory proteins [5,31]. Cysteine scanning accessibility analysis was used to determine the transmembrane topology of the protein [32]. NHE1 was found to have 12 transmembrane segments and three membrane associated loop regions (Fig. 2). NHE1 has both *N*- and *O*-linked glycosylation sites. *N*-linked glycosylation can be removed without adversely affecting transport [33,34]. It was originally suggested that the first transmembrane segment of NHE1 was not a cleavable signal sequence while that of NHE3 is cleaved [31,35]. Later the first segment of NHE6 and also that of NHE1 was suggested to be cleavable as a signal sequence [36]. NHE1 forms dimers in the membrane [37–39].

An interesting aspect of the NHE1 isoform of the Na^+/H^+ exchanger that has implications in cardiovascular biology is the involvement of the NHE1 gene in sodium–lithium counter transport. Na^+/Li^+ exchange is a well-known activity of erythrocytes where it occurs with a one to one stoichiometry [40]. Elevated activity of the sodium lithium exchanger is a good predictor of development of essential hypertension and diabetic nephropathy and increased Na^+/Li^+ exchange occurs in several tissue types though it is not clear whether this elevated activity is symptomatic or causal in this disease [41–45]. In the past, it was thought that a completely different protein than NHE1 must mediate Na^+/Li^+ exchange, as it was insensitive to inhibitors of NHE1 [46,47]. However, alternative splicing of human NHE1 was shown to yield a protein with the capability of transport of Na^+ for Li^+ and which was virtually unable to mediate exchange of Na^+ for H^+ [47]. Three transmembrane segments of NHE1 are eliminated by the splicing event including transmembrane segment IV, which is involved in mediating sensitivity to inhibition by various compounds (Fig. 3A, see also Section 3 below) [47,48]. This likely accounts for the lack of susceptibility to inhibition by NHE1 inhibitors. While genetic linkage analysis suggests that the NHE1 gene is not a direct candidate for essential hypertension, this may not preclude an involvement of regulators affecting NHE1 function [49]. How the NHE1 protein mediates a Na^+/Li^+ exchange as opposed to Na^+/H^+ exchange remains unknown.

3. Mechanism of Na^+/H^+ exchange activity and inhibition

The Na^+/H^+ exchanger is involved in a number of cardiac diseases and its inhibition with specific inhibitors is under investigation for prevention of cardiac disease (see above). Therefore, the understanding of how the protein functions and where inhibitors bind and how they act are of great interest scientifically and could lead to the development of novel NHE1 inhibitors that might prove useful in the clinic. The membrane domain of the Na^+/H^+ exchanger is responsible for transport of cations across the membrane. How it mediates cation transport is still under investigation. It has been suggested that a few critical amino acids of the membrane domain of NHE1 are involved in cation coordination and transport [1]. The complete details of how this occurs likely await the completion of a combination of studies on the details of the structure of the protein and of studies identifying amino acids critical in transport. The structure of the membrane domain of the eukaryotic protein has not been deduced but is of interest, especially since experiments with domain swapping and site-specific mutagenesis have shown that the membrane domain

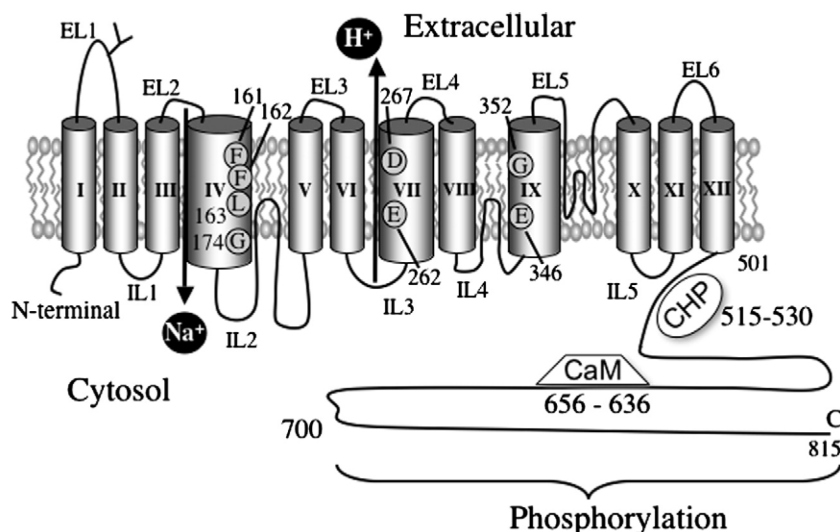

Fig. 2. Two-dimensional topology of the Na^+/H^+ exchanger. Transmembrane segments are labeled I to XII, EL1-EL6 are extracellular loops one to six, IL1-IL5 are intracellular loops one to five. Transmembrane segments IV, VII and IX are enlarged for illustrative purposes only. Amino acids important in transport and/or affecting inhibitor efficacy are illustrated. Regions involved in binding calmodulin (CaM) and calcineurin homologous protein (CHP) are shown. The region of the cytosolic domain involved in phosphorylation and regulation of activity is indicated.

Author's personal copy

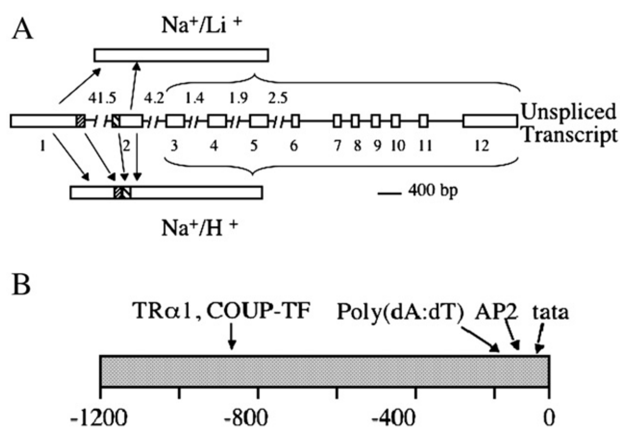

Fig. 3. NHE1 promoter structure and regulation. (A) Intron–exon structure of the NHE1 promoter. The unspliced transcript is indicated. Boxes indicate exons while lines indicate introns. Exon numbers are indicated below the unspliced transcript. Sizes of the first 5 introns are indicated above the unspliced transcript. Regions of exon 1 and 2 that are differentially spliced are indicated by cross hatching. Inclusion of these regions results in the Na^+/H^+ exchanger. Exclusion of them results in a Na^+/Li^+ exchanger. (B) Schematic diagram of the initial 1200 bp of the mouse NHE1 promoter. Arrows indicate the approximate location of binding sites for the transcription factors shown to be important in regulation of the gene in the myocardium.

is the site of binding of inhibitors of the protein [50,51]. The Na^+ binding site and the NHE1 inhibitor site are related and may be overlapping but not identical in the protein [52]. Mutating residues in transmembrane segments IV, VII and IX has revealed alterations in affinity for NHE1 inhibitors. This mostly causes a decrease in the efficacy of inhibitors and sometimes, but not always, is accompanied by changes in the affinity for sodium. Transmembrane segment IV has some startling effects with mutations in Phe165 of hamster NHE1 (Phe161 in human NHE1) causing both an increase in resistance to inhibitors and a decrease in V_{max} for Na^+ [53]. Phe162, Leu163 and Gly174 also cause large changes of up to over 1000-fold in K_i [50,53,54]. In some of the other transmembrane segments including VII and IX and in extracellular loops of the protein, similar though smaller effects have been shown in other amino acids while mutations to some critical amino acids totally eliminate NHE1 function (Fig. 2). These include amino acids Gly148, Pro167/Pro168, Glu262, Asp267, Glu346 and Gly352 [55–58]. While it is clear that these amino acids affect the sensitivity to the inhibitors and it is likely that they are close to the binding site of the inhibitors, the exact location of NHE1 inhibitor binding is not yet known and awaits elucidation.

The complete structure of the NHE1 protein has not yet been elucidated. The general topology of the protein is known (Fig. 2); however, knowledge of the three dimensional structure is lacking. A Na^+/H^+ exchanger protein from *E. coli*, NhaA has been purified and the structure deduced [59]. It has 12 membrane-spanning segments that are oriented so as to form a negatively charged funnel that opens to the cytosol. In the middle of the membrane is a putative ion-binding site. Ten of the transmembrane segments are alpha helices while two, IV and XI, have only partial helical character with extended regions that form part of the ion binding site. It has recently been proposed that three conserved aspartates are critical to Na^+/H^+ antiport in NhaA. One amino acid (*Asp163*) is believed to bind Na^+, another (Asp164) to control accessibility to this site from either side of the membrane while a third (D133) is thought to be crucial for pH regulation [60]. These observations support the theory that a few critical amino acids of NHE1 are involved in cation coordination and transport [1]. While the entire structure of NHE1 is not known, the structure of several transmembrane segments has been elucidated. Transmembrane segment IV has partial helical character with an extended region in the middle [61]. It is similar in general structure to transmembrane segment IV of NhaA suggesting it may also be important in ion coordination [48]. Several other studies also support a critical role for transmembrane segment IV. These include site-specific mutagenesis that shows that amino acids in this region are critical to the transport function of the protein [53,62]. In addition, Phe161 has been shown to be a pore-lining residue by cysteine scanning mutagenesis analysis [61].

The structure of transmembrane segment VII of NHE1 has been elucidated. It is predominantly alpha helical with a break near the middle of the helix at functionally important residues [58]. Several residues of the membrane segment have been shown to be critical to transport and affect ion coordination and transport though it is uncertain whether they function in the same manner as Asp163 and Asp164 of NhaA [56]. L255 and L258 are affected by sulfhydryl reactive reagents when mutated to cysteine residues suggesting they may be pore-lining residues [63]. It is therefore very likely that transmembrane segment VII is involved in cation coordination and transport.

A variety of other amino acids are known to be critical to NHE1 function. These include amino acids of extracellular loop 2 and intracellular loop 2 [32,55]. Additionally, mutations in transmembrane segment XI are known to affect the affinity of the protein for protons [64]. Clearly these regions are important in the function of the protein, though further elucidation of their role in transport and how they function in the structure of the intact protein, is called for. A greater understanding of the structure and function of the NHE1 protein is of significant cardiovascular interest since it could lead to improved and more specific NHE1 inhibitors. Recently, in a clinical trial with an NHE1 inhibitor, significant side effects were shown. While administration of the NHE1 inhibitor cariporide produced a significant decrease in risk in coronary artery bypass patients, there was an increase in the overall rate of cerebrovascular events in the treated group [65]. Whether this was due to lack of specificity of the NHE1 inhibitor is not known, and further investigation in this area seems warranted.

Regulation of the Na^+/H^+ exchanger has also been the subject of a large number of studies. Na^+/H^+ exchanger is mainly quiescent until activated by decreases in intracellular pH. Decreasing intracellular pH causes a rapid activation with a Hill coefficient of approximately 3, indicative of a proton modifier site that is suggested to aid in rapid activation of the protein [48]. The activity of the membrane domain is regulated through the large cytosolic domain. The factors that affect the activation of the protein by intracellular pH include phosphorylation of the cytosolic domain and interaction of the cytosolic

Author's personal copy

domain with other regulatory molecules. Phosphorylation in the distal region of the tail amino acids 700–815 (Fig. 2) shifts the activation of the protein such that it is more active at more alkaline intracellular pHs [66]. A number of protein kinases are implicated depending on the tissue. In the myocardium, ERK1/2 and $p90^{rsk}$ (p90ribosomalS6kinase) may be important [67,68]. Both acidosis and myocardial ischemia have been shown to activate these regulatory pathways [69–71]. A number of other proteins interact with the cytosolic domain and also modify NHE1 activity. The best known of these is calmodulin. Calmodulin binds to a high affinity site located at amino acids 636–656 and regulates NHE1 activity by blocking this autoinhibitory region [72,73]. What happens to calmodulin binding and this autoregulatory region in pathological conditions including calcium overload is not known. Another calcium binding regulator protein is CHP (calcineurin homologous protein). It binds to amino acids 515–530, promotes NHE1 activity and is thought to be an essential cofactor for activity [74]. Whether its role in NHE1 regulation changes in cardiac disease is not known, and it has not been studied in the myocardium. Other regulatory factors that bind to NHE1 are carbonic anhydrase II, PIP2 and heat shock protein 70; however, their role in the myocardium has not been well studied [75–77].

4. NHE1 expression in the myocardium

The type 1 isoform of the Na^+/H^+ exchanger has long been known to be induced by acidosis in a number of tissues. Early studies examined NHE1 expression in the kidney. It was shown that treatment of renal cell lines with acidosis increased NHE1 mRNA, activity and protein levels [78,79]. Chronic acidosis of treated animals also showed that NHE1 expression was elevated in the kidney [80]. Similar results were shown in some but not all cell types examined under conditions of metabolic acidosis [81,82]. However, it was not until later that regulation of expression in myocardial tissues was examined. Isolated cardiomyocytes subjected to chronic external acidosis, were shown to have elevated NHE1 activity and treatment of isolated perfused hearts with ischemia elevated NHE1 mRNA levels [83,84]. Coronary artery ligation in rats also caused elevation of NHE1 mRNA levels; thus clearly, acidosis and various types of cardiovascular stress can elevate NHE1 mRNA levels in the heart [85].

A number of studies have shown that NHE1 is involved in mediating heart hypertrophy and blockage of NHE1 activity can prevent hypertrophy [86–91]. Different evidence supports the causal relationship between hypertrophic factors and NHE1. For example, NHE1 is activated by MAP kinases and protein kinase C-dependent pathways, which are important in hypertrophic and remodeling processes [71,92]. In addition, the effect of the hypertrophic agonists such as norepinephrine and aldosterone can be blocked by NHE1 inhibition as can stretch induced hypertrophy [91–93].

How NHE1 inhibition prevents myocardial hypertrophy is under investigation. Enhanced activity of NHE1 is not accompanied by intracellular alkalosis in the spontaneously hypertensive rat and in medium containing bicarbonate, angiotension and endothelin-1 agonists of NHE1, do not increase intracellular pH [94,95]. Thus, NHE1 inhibition may not act through prevention of elevation of intracellular pH. Prevention of increases in intracellular Na^+ is a good candidate for a mechanism by which NHE1 inhibition prevents hypertrophy [95]. This could act through elevation of reverse mode of Na^+/Ca^{2+} exchanger activity through the increased intracellular Na^+. A role for this pathway has been implicated in hypertrophy induced by endothelin-1 and blocked by NHE1 inhibition [96]. However, Na^+/Ca^{2+} knockout mice still demonstrate hypertrophy in a model of aortic constriction suggesting that the Na^+/Ca^{2+} is not critical in all models of hypertrophy that are treatable with NHE1 inhibitors [97]. Elevation of intracellular Na^+ could act through activation of intracellular signaling pathways such as protein kinase C and elevated intracellular Na^+ may also increase reactive oxygen species that activate ERK1/2-dependent phosphorylation pathways [98,99]. In this regard, NHE1 inhibition has been demonstrated to prevent detrimental phenylephrine induced effects on mitochondria including prevention of mitochondrial permeability transition pore opening, loss of mitochondrial membrane potential, activation of p38 map kinases and ERK1/2 and reduction of mitochondria derived reactive oxygen species [100]. Calcineurin is a prohypertrophic protein phosphatase and one report has also shown that the NHE1 inhibitor cariporide, decreased calcineurin levels [101].

While it is clear that NHE plays an important role in myocardial hypertrophy, an interesting and likely complicating component of this is that NHE1 expression is increased in myocardial hypertrophy. For example, elevated NHE1 mRNA and protein levels have been demonstrated for β_1-adrenergic receptor transgenic mice that develop hypertrophy, for NHE1 mRNA in monocrotaline-induced ventricular hypertrophy, for NHE1 mRNA in diabetes induced vascular hypertrophy and for NHE1 mRNA and protein in aldosterone induced hypertrophy [87,91,102,103]. The mechanism by which these increases occur is not known and the role of elevated NHE1 levels in mediating hypertrophy has yet to be investigated.

It is well known that in cardiac hypertrophy induced by pressure overload there is a switch in cardiac gene expression towards a more fetal program of gene expression [104,105]. NHE1 expression in the myocardium is also regulated such that activity of the promoter is high during early embryonic development and the NHE1 protein is at relatively high levels in the neonate and declines with age [106–109]. It may be that, similar to effects on the myosin heavy chain, the switch to the fetal type of gene expression during hypertrophy causes the activity of the NHE1 promoter to be increased by a similar mechanism. Since greater levels of the NHE1 protein may predispose the myocardium to even more hypertrophy, future studies might examine how the elevation of NHE1 levels is important in heart disease [9,86–91].

5. The NHE1 gene

The NHE1 gene has been cloned and characterized from several species. This includes the human, rabbit, porcine and

Author's personal copy

mouse forms of the promoter [110–113]. Analysis of the human gene showed that it contains 12 exons and 11 introns (Fig. 3A). The mouse NHE1 promoter has a similar design [114]. The first exon is separated from the second exon by a very large (41.5 kb) intron while the other introns are much smaller and vary in length from 4.2 to 0.37 kb [110]. Several studies examined the transcription factors involved in regulation of the gene, mostly in tissues outside the myocardium. Briefly described, the transcription factor AP-1 has been shown to be important in expression in renal proximal cells while the C/EBP family of transcription factors was suggested to be an important regulator in NIH 3T3 cells (fibroblasts), in vascular smooth muscle A7r5 cells and in hepatic (HepG2) cells [115–117]. Both are in relatively proximal regions of the gene, near the transcriptional start site. Analysis of the activity of the rabbit promoter demonstrated that a negative regulatory element exists further upstream of the 1.1-kb proximal region, that has been more typically studied [111]. Both the porcine and the rabbit NHE1 promoter are homologous to the human and mouse promoters particularly in the proximal 500 bp of the 5′-flanking region. Several consensus elements for the transcription factors AP-1, C/EBP and Sp1 are conserved between pig and human while AP3 and PEA3 are only found in the porcine promoter [112]. In the mouse NHE1 promoter, a proximal AP-2 binding site is important in expression in fibroblasts and in P19 embryonal carcinoma cells [83,113]. Differentiation of P19 cells or L6 muscle cells causes up to a 10-fold induction in the NHE1 promoter that is dependent on the proximal AP-2 site [118,119]. Several other regions of the NHE1 promoter are important in a variety of cells. A conserved poly (dA:dT) region of the NHE1 promoter is located at bp −155 to −169 of the mouse gene and is important in L6 and NIH 3T3 cells [120]. Chicken ovalbumin upstream promoter transcription factor (COUP-TF) types I and II is a somewhat more distal (−841 to −800 bp) factor important in expression [121]. In this same region, the thyroid hormone receptor $TR\alpha_1$ is also implicated in regulation of the promoter [122].

Studies directly examining expression of the NHE1 promoter in the myocardium have been limited in number. Regions demonstrated to be important in NHE1 promoter activity in the myocardium are indicated in Fig. 3B. Using isolated cardiomyocytes, we examined a 1.1-kb region of the mouse promoter. Serum stimulated activity of the promoter in cardiomyocytes and with deletion of the AP-2 site (bp −95 to −111), there was a 4-fold decrease in NHE1 promoter activity compared with the intact gene [123]. Mutation of the AP-2 site, combined with deletion of distal regions of the promoter, almost totally eliminated promoter activity in cardiomyocytes. Another region, a poly(dA:dT) rich region (−155 to −169), is protected by heart nuclear extracts in DNase I footprinting analysis as is the COUP-TF element in the −841 to −800 region [124]. Thyroid hormone is also suggested to regulate the NHE1 gene in the myocardium. Protein binding in the COUP-TF region is increased with treatment of heart cells with thyroid hormone and treatment of cardiomyocytes with thyroid hormone increases NHE1 protein expression [122]. Further studies are necessary to learn what other regions are important in regulation of expression in the myocardium particularly in disease states.

It is of interest and relevant to the myocardium that recent studies have shown that the NHE1 promoter is responsive to reactive oxygen species (ROS). ROS have been shown to be critical mediators of growth-promoting signaling events involved in the hypertrophic pathways in muscle cells [125,126]. The role of ROS in ET-1-induced cardiac hypertrophy has been further confirmed by studies showing that ET-1-mediated generation of ROS in cardiac hypertrophy can be inhibited by pretreatment with an antioxidant [126–128]. With regard to the NHE1 promoter, it was shown that increasing serum from 0.5 to 10% induces NHE1 promoter activity in NIH3T3 fibroblasts. This increase correlated with O_2 superoxide production and both O_2 superoxide production and NHE1 promoter activity could be blocked by the oxidase inhibitor diphenyleniodonium [129]. The effect of O_2 superoxide on gene expression was further supported by the ability of tiron, a specific O_2 superoxide scavenger to revert increases in NHE1 promoter activity and protein levels [129]. These results suggested that the NHE1 gene and protein expressions are targeted by O_2 superoxide. The same phenomenon that occurred in human glioma cells were diphenyleniodonium was also shown to reduce NHE1 protein expression [129,130]. Since NHE1 levels have been shown to be elevated in the myocardium following ischemic heart disease, it may be that the mechanism of elevated NHE1 expression is through the effects of O_2 superoxide on the NHE1 promoter. Future experiments may examine this phenomenon.

6. Conclusions

Our understanding of the Na^+/H^+ exchanger protein has progressed greatly since the initial cloning of the cDNA for the human NHE1 isoform. We now have important information on some of the amino acids involved in transport and the beginnings of an understanding of the protein's structure. It is clear that the NHE1 isoform plays an important role in the myocardium in heart disease. Though great success was found in animal studies on the use of NHE1 inhibitors to treat ischemia reperfusion damage, these have not yet been translated to the bedside. A better understanding of how the protein functions and how inhibition of activity of the protein occurs could lead to improved NHE1 inhibition in the clinical setting. Why the NHE1 protein message and activity is upregulated in some forms of heart disease may also lead to a better understanding of how the protein influences myocardial hypertrophy. Great progress has been made, but many areas remain to yet be elucidated.

Acknowledgments

L.F. was supported by an Alberta Heritage Foundation for Medical Research Scientist Award. Research by L.F. in this area is supported by the CIHR.

Author's personal copy

References

[1] Dibrov P, Fliegel L. Comparative molecular analysis of Na^+/H^+ exchangers: a unified model for Na^+/H^+ antiport? FEBS Lett 1998; 424:1–5.

[2] Lagadic-Gossmann D, Buckler KJ, Vaughan-Jones RD. Role of bicarbonate in pH recovery from intracellular acidosis in the guinea-pig ventricular myocyte. J Physiol 1992;458:361–84.

[3] Lagadic-Gossmann D, Vaughan-Jones RD, Buckler KJ. Adrenaline and extracellular ATP switch between two modes of acid extrusion in the guinea-pig ventricular myocyte. J Physiol 1992;458:385–407.

[4] Grace AA, Kirschenlohr HL, Metcalfe JC, Smith GA, Weissberg PL, Cragoe Jr EJ, et al. Regulation of intracellular pH in the perfused heart by external HCO3− and Na(+)–H+ exchange. Am J Physiol 1993;265: H289–98.

[5] Malo ME, Fliegel L. Physiological role and regulation of the Na^+/H^+ exchanger. Can J Physiol Pharmacol 2006;84:1081–95.

[6] Avkiran M, Haworth RS. Regulatory effects of G protein-coupled receptors on cardiac sarcolemmal Na^+/H^+ exchanger activity: signaling and significance. Cardiovasc Res 2003;57:942–52.

[7] Avkiran M, Marber MS. Na(+)/H(+) exchange inhibitors for cardioprotective therapy: progress, problems and prospects. J Am Coll Cardiol 2002;39:747–53.

[8] Phillis JW, Pilitsis JG, O'Regan MH. The potential role of the Na^+/H^+ exchanger in ischemia/reperfusion injury of the central nervous system. p. 177–189. In: Karmazyn M, Avkiran M, Fliegel L, editors. The Na^+/H^+ Exchanger, From Molecular to Its Role in Disease. Boston: Kluwer Academic Publishers; 2003. 318 pp.

[9] Karmazyn M. Therapeutic potential of Na^+–H^+ exchange inhibitors for the treatment of heart failure. Expert Opin Investig Drugs 2001;10: 835–43.

[10] Gazmuri RJ, Hoffner E, Kalcheim J, Ho H, Patel M, Ayoub IM, et al. Myocardial protection during ventricular fibrillation by reduction of proton-driven sarcolemmal sodium influx. J Lab Clin Med 2001;137: 43–55.

[11] Theroux P, Chaitman BR, Danchin N, Erhardt L, Meinertz T, Schroeder JS, et al. Inhibition of the sodium–hydrogen exchanger with cariporide to prevent myocardial infarction in high-risk ischemic situations. Main results of the GUARDIAN trial. Guard during ischemia against necrosis (GUARDIAN) investigators. Circulation 2000;102:3032–8.

[12] Zeymer U, Suryapranata H, Monassier JP, Opolski G, Davies J, Rasmanis G, et al. The Na(+)/H(+) exchange inhibitor eniporide as an adjunct to early reperfusion therapy for acute myocardial infarction. Results of the evaluation of the safety and cardioprotective effects of eniporide in acute myocardial infarction (ESCAMI) trial. J Am Coll Cardiol 2001;38: 1644–50.

[13] Sardet C, Franchi A, Pouysségur J. Molecular cloning, primary structure, and expression of the human growth factor-activatable Na^+/H^+ antiporter. Cell 1989;56:271–80.

[14] Frohlich O. The NHE family of Na^+/H^+ exchangers; Its known and putative members and what can be learned by comparing them with each other. In: Fliegel L, editor. The Na^+/H^+ Exchanger. R.G. Landes Company; 1996. p. 295–307.

[15] Fliegel L, Sardet C, Pouysségur J, Barr A. Identification of the protein and cDNA of the cardiac Na^+/H^+ exchanger. FEBS Lett 1991;279:25–9.

[16] Fliegel L, Dyck JRB, Wang H, Fong C, Haworth RS. Cloning and analysis of the human myocardial Na^+/H^+ exchanger. Mol Cell Biochem 1993;125:137–1343.

[17] Janecki AJ, Janecki M, Akhter S, Donowitz M. Quantitation of plasma membrane expression of a fusion protein of Na^+/H^+ exchanger NHE3 and green fluorescence protein (GFP) in living PS120 fibroblasts. J Histochem Cytochem 2000;48:1479–92.

[18] Orlowski J, Kandasamy RA, Shull GE. Molecular cloning of putative members of the Na^+/H^+ exchanger gene family. J Biol Chem 1992;267: 9331–9.

[19] Noel J, Roux D, Pouyssegur J. Differential localization of Na^+/H^+ exchanger isoforms (NHE1 and NHE3) in polarized epithelial cell lines. J Cell Sci 1996;109:929–39.

[20] Attaphitaya S, Park K, Melvin JE. Molecular cloning and functional expression of a rat Na^+/H^+ exchanger (NHE5) highly expressed in brain. J Biol Chem 1999;274:4383–8.

[21] Baird NR, Orlowski J, Szabo EZ, Zaun HC, Schultheis PJ, Menon AG, et al. Molecular cloning, genomic organization, and functional expression of Na^+/H^+ exchanger isoform 5 (NHE5) from human brain. J Biol Chem 1999;274:4377–82.

[22] Wang Z, Orlowski J, Shull GE. Primary structure and functional expression of a novel gastrointestinal isoform of the rat Na^+/H^+ exchanger. J Biol Chem 1993;268:11925–8.

[23] Brant SR, Yun CHC, Donowitz M, Tse CM. Cloning, tissue distribution, and functional analysis of the human Na^+/H^+ exchanger isoform, NHE3. Am J Physiol 1995;269:C198–206.

[24] Colombani V, Silviani V, Marteau C, Lerique B, Cartouzou G, Gerolami A. Presence of the NHE3 isoform of the Na^+/H^+ exchanger in human gallbladder. Clin Sci (Lond) 1996;91:209–12.

[25] Pizzonia JH, Biemesderfer D, Abu-Alfa AK, Wu MS, Exner M, Isenring P, et al. Immunochemical characterization of Na^+/H^+ exchanger isoform NHE4. Am J Physiol 1998;275:F510–7.

[26] Nakamura N, Tanaka S, Teko Y, Mitsui K, Kanazawa H. Four Na^+/H^+ exchanger isoforms are distributed to Golgi and post-Golgi compartments and are involved in organelle pH regulation. J Biol Chem 2005;280: 1561–72.

[27] Goyal S, Vanden Heuvel G, Aronson PS. Renal expression of novel Na^+/H^+ exchanger isoform NHE8. Am J Physiol 2003;284:F467–73.

[28] Numata M, Petrecca K, Lake N, Orlowski J. Identification of a mitochondrial Na^+/H^+ exchanger. J Biol Chem 1998;273:6951–9.

[29] Brett CL, Wei Y, Donowitz M, Rao R. Human Na(+)/H(+) exchanger isoform 6 is found in recycling endosomes of cells, not in mitochondria. Am J Physiol 2002;282:C1031–41.

[30] Numata M, Orlowski J. Molecular cloning and characterization of a novel $(Na^+,K^+)/H^+$ exchanger localized to the trans-Golgi network. J Biol Chem 2001;276:17387–94.

[31] Wakabayashi S, Fafournoux P, Sardet C, Pouyssegur J. The Na^+/H^+ antiporter cytoplasmic domain mediates growth factor signals and controls "H(+)-sensing". Proc Natl Acad Sci U S A 1992;89:2424–8.

[32] Wakabayashi S, Pang T, Su X, Shigekawa M. A novel topology model of the human Na^+/H^+ exchanger isoform 1. J Biol Chem 2000;275: 7942–9.

[33] Counillon L, Pouyssegur J, Reithmeier RA. The Na^+/H^+ exchanger NHE-1 possesses *N*- and *O*-linked glycosylation restricted to the first N-terminal extracellular domain. Biochemistry 1994;33:10463–9.

[34] Haworth RS, Frohlich O, Fliegel L. Multiple carbohydrate moieties on the Na^+/H^+ exchanger. Biochem J 1993;289:637–40.

[35] Zizak M, Cavet ME, Bayle D, Tse CM, Hallen S, Sachs G, et al. Na(+)/H(+) exchanger NHE3 has 11 membrane spanning domains and a cleaved signal peptide: topology analysis using in vitro transcription/ translation. Biochemistry 2000;39:8102–12.

[36] Miyazaki E, Sakaguchi M, Wakabayashi S, Shigekawa M, Mihara K. NHE6 protein possesses a signal peptide destined for endoplasmic reticulum membrane and localizes in secretory organelles of the cell. J Biol Chem 2001;276:49221–7.

[37] Fliegel L, Haworth RS, Dyck JRB. Characterization of the placental brush border membrane Na^+/H^+ exchanger: identification of thiol-dependent transitions in apparent molecular size. Biochem J 1993;289:101–7.

[38] Fafournoux P, Noel J, Pouysségur J. Evidence that Na^+/H^+ exchanger isoforms NHE1 and NHE3 exist as stable dimers in membranes with a high degree of specificity for homodimers. J Biol Chem 1994;269: 2589–96.

[39] Hisamitsu T, Pang T, Shigekawa M, Wakabayashi S. Dimeric interaction between the cytoplasmic domains of the Na^+/H^+ exchanger NHE1 revealed by symmetrical intermolecular cross-linking and selective co-immunoprecipitation. Biochemistry 2004;43:11135–43.

[40] Haas M, Schooler J, Tosteson DC. Coupling of lithium to sodium transport in human red cells. Nature 1975;258:425–7.

[41] Williams RR, Hunt SC, Kuida H, Smith JB, Ash KO. Sodium–lithium countertransport in erythrocytes of hypertension prone families in Utah. Am J Epidemiol 1983;118:338–444.

Author's personal copy

[42] Hasstedt SJ, Wu LL, Ash KO, Kuida H, Williams RR. Hypertension and sodium–lithium countertransport in Utah pedigrees: evidence for major-locus inheritance. Am J Hum Genet 1988;43:14–22.

[43] Monciotti CG, Semplicini A, Morocutti A, Maioli M, Cipollina MR, Barzon I, et al. Elevated sodium–lithium countertransport activity in erythrocytes is predictive of the development of microalbuminuria in IDDM. Diabetologia 1997;40:654–61.

[44] Canessa M, Adragna N, Solomon HS, Connolly TM, Tosteson DC. Increased sodium–lithium countertransport in red cells of patients with essential hypertension. N Engl J Med 1980;302:772–6.

[45] Zerbini G, Podesta F, Meregalli G, Deferrari G, Pontremoli R. Fibroblast Na^+–Li^+ countertransport rate is elevated in essential hypertension. J Hypertens 2001;19:1263–9.

[46] Kahn AM. Difference between human red blood cell Na^+–Li^+ countertransport and renal Na^+–H^+ exchange. Hypertension 1987;9:7–12.

[47] Zerbini G, Maestroni A, Breviario D, Mangili R, Casari G. Alternative splicing of NHE-1 mediates Na–Li countertransport and associates with activity rate. Diabetes 2003;52:1511–8.

[48] Slepkov ER, Rainey JK, Sykes BD, Fliegel L. Structural and functional analysis of the Na(+)/H(+) exchanger. Biochem J 2007;401:623–33.

[49] Lifton RP, Hunt SC, Williams RR, Pouyssegur J, Lalouel JM. Exclusion of the Na(+)–H+ antiporter as a candidate gene in human essential hypertension. Hypertension 1991;17:8–14.

[50] Counillon L, Noel J, Reithmeier RAF, Pouyssegur J. Random mutagenesis reveals a novel site involved in inhibitor interaction within the fourth transmembrane segment of the Na^+/H^+ exchanger-1. Biochemistry 1997;36:2951–9.

[51] Orlowski J, Kandasamy RA. Delineation of transmembrane domains of the Na^+/H^+ exchanger that confer sensitivity to pharmacological antagonists. J Biol Chem 1996;271:19922–7.

[52] Harris C, Fliegel L. Amiloride and the Na^+/H^+ exchanger protein. Mechanism and significance of inhibition of the Na^+/H^+ exchanger. Int J Mol Med 1999;3:315–21.

[53] Counillon L, Franchi A, Pouyssegur J. A point mutation of the Na^+/H^+ exchanger gene (NHE1) and amplification of the mutated allele confer amiloride resistance upon chronic acidosis. Proc Natl Acad Sci U S A 1993;90:4508–12.

[54] Touret N, Poujeol P, Counillon L. Second-site revertants of a low-sodium-affinity mutant of the Na^+/H^+ exchanger reveal the participation of TM4 into a highly constrained sodium-binding site. Biochemistry 2001;40:5095–101.

[55] Khadilkar A, Iannuzzi P, Orlowski J. Identification of sites in the second exomembrane loop and ninth transmembrane helix of the mammalian Na^+/H^+ exchanger important for drug recognition and cation translocation. J Biol Chem 2001;276:43792–800.

[56] Murtazina R, Booth BJ, Bullis BL, Singh DN, Fliegel L. Functional analysis of polar amino-acid residues in membrane associated regions of the NHE1 isoform of the mammalian Na^+/H^+ exchanger. Eur J Biochem 2001;268:4674–85.

[57] Noel J, Germain D, Vadnais J. Glutamate 346 of human Na^+–H^+ exchanger NHE1 is crucial for modulating both the affinity for Na^+ and the interaction with amiloride derivatives. Biochemistry 2003;42:15361–8.

[58] Ding J, Rainey JK, Xu C, Sykes BD, Fliegel L. Structural and functional characterization of transmembrane segment VII of the Na^+/H^+ exchanger isoform 1. J Biol Chem 2006;281:29817–29.

[59] Hunte C, Screpanti E, Venturi M, Rimon A, Padan E, Michel H. Structure of a Na^+/H^+ antiporter and insights into mechanism of action and regulation by pH. Nature 2005;435:1197–202.

[60] Arkin IT, Xu H, Jensen MO, Arbely E, Bennett ER, Bowers KJ, et al. Mechanism of Na^+/H^+ antiporting. Science 2007;317:799–803.

[61] Slepkov ER, Rainey JK, Li X, Liu Y, Cheng FJ, Lindhout DA, et al. Structural and functional characterization of transmembrane segment IV of the NHE1 isoform of the Na^+/H^+ exchanger. J Biol Chem 2005;280:17863–72.

[62] Slepkov ER, Chow S, Lemieux MJ, Fliegel L. Proline residues in transmembrane segment IV are critical for activity, expression and targeting of the Na^+/H^+ exchanger isoform 1. Biochem J 2004;379:31–8.

[63] Ding J, Ng RWP, Fliegel L. Functional characterization of the transmembrane segment VII of the NHE1 isoform of the Na^+/H^+ exchanger. Can J Physiol Pharmacol 2007;85:319–25.

[64] Wakabayashi S, Hisamitsu T, Pang T, Shigekawa M. Mutations of Arg440 and Gly455/Gly456 oppositely change pH sensing of Na^+/H^+ exchanger 1. J Biol Chem 2003;278:11828–35.

[65] Mentzer Jr RM. Effects of Na^+/H^+ exchange inhibition by cariporide on death and nonfatal myocardial infarction in patients undergoing coronary artery bypass graft surgery: The Expedition Study. Circulation 2003;108(3M):2723 (Abstract).

[66] Sardet C, Fafournoux P, Pouyssegur J. Alpha-thrombin, epidermal growth factor, and okadaic acid activate the Na^+/H^+ exchanger, NHE-1, by phosphorylating a set of common sites. J Biol Chem 1991;266:19166–71.

[67] Moor AN, Fliegel L. Protein kinase mediated regulation of the Na^+/H^+ exchanger in the rat myocardium by MAP-kinase-dependent pathways. J Biol Chem 1999;274:22985–92.

[68] Maekawa N, Abe J, Shishido T, Itoh S, Ding B, Sharma VK, et al. Inhibiting p90 ribosomal S6 kinase prevents (Na^+)–H^+ exchanger-mediated cardiac ischemia-reperfusion injury. Circulation 2006;113:2516–23.

[69] Haworth RS, McCann C, Snabaitis AK, Roberts NA, Avkiran M. Stimulation of the plasma membrane Na^+/H^+ exchanger NHE1 by sustained intracellular acidosis. Evidence for a novel mechanism mediated by the ERK pathway. J Biol Chem 2003;278:31676–84.

[70] Malo ME, Li L, Fliegel L. Mitogen-activated protein kinase-dependent activation of the Na^+/H^+ exchanger is mediated through phosphorylation of amino acids Ser770 and Ser771. J Biol Chem 2007;282:6292–9.

[71] Moor A, Gan XT, Karmazyn M, Fliegel L. Protein kinase mediated regulation of the Na^+/H^+ exchanger isoform 1 (NHE1) in ischemic and ischemic-reperfused rat heart. J Biol Chem 2001;27:16113–22.

[72] Wakabayashi S, Bertrand B, Ikeda T, Pouyssegur J, Shigekawa M. Mutation of calmodulin-binding site renders the Na^+/H^+ exchanger (NHE1) highly H^+-sensitive and Ca^{2+} regulation-defective. J Biol Chem 1994;269:13710–5.

[73] Bertrand B, Wakabayashi S, Ikeda T, Pouyssegur J, Shigekawa M. The Na^+/H^+ exchanger isoform 1 (NHE1) is a novel member of the calmodulin-binding proteins. J Biol Chem 1994;269:13703–9.

[74] Pang T, Su X, Wakabayashi S, Shigekawa M. Calcineurin homologous protein as an essential cofactor for Na^+/H^+ exchangers. J Biol Chem 2001;276:17367–72.

[75] Li X, Alvarez B, Casey JR, Reithmeier RA, Fliegel L. Carbonic anhydrase II binds to and enhances activity of the Na^+/H^+ exchanger. J Biol Chem 2002;277:36085–91.

[76] Aharonovitz O, Zaun HC, Balla T, York JD, Orlowski J, Grinstein S. Intracellular pH regulation by Na(+)/H(+) exchange requires phosphatidylinositol 4,5-bisphosphate. J Cell Biol 2000;150:213–24.

[77] Silva NLCL, Haworth RS, Singh D, Fliegel L. The carboxyl-terminal region of the Na^+/H^+ exchanger interacts with mammalian heat shock protein. Biochemistry 1995;34:10412–20.

[78] Kinsella J, Cujdik T, Sacktor B. Na^+–H^+ exchange activity in renal brush border membrane vesicle in response to metabolic acidosis: the role of glucocorticoids. Proc Natl Acad Sci U S A 1984;81:630–4.

[79] Mrkic B, Helmle-Kolib C, Krapf R, Murer H. Functional adaptation to high PCO2 of apically and basolaterally located Na^+/H^+ exchange activities in cultured renal cell lines. Pflugers Arch 1994;H426:333–40.

[80] Krapf R, Pearce D, Lynch C, Xi XP, Reudelhuber TL, Pouyssegur J, et al. Expression of rat renal Na^+/H^+ antiporter mRNA levels in response to respiratory and metabolic acidosis. J Clin Invest 1991;87:747–51.

[81] Moe OW, Miller RT, Horie S, Cano A, Preisig PA, Alpern RJ. Differential regulation of Na/H antiporter by acid in renal epithelial cells and fibroblasts. J Clin Invest 1991;88:1703–8.

[82] Quednau B, Rosskopf D, Reusch HP, Luft FC, Siffert W. Enhanced Na^+/H^+ exchanger activity and NHE1 mRNA levels in human lymphocytes during metabolic acidosis. Am J Physiol 1994;266:C480–8.

[83] Dyck JRB, Maddaford T, Pierce GN, Fliegel L. Induction of expression of the sodium–hydrogen exchanger in rat myocardium. Cardiovasc Res 1995;29:203–8.

Author's personal copy

[84] Gan XT, Chakrabarti S, Karmazyn M. Modulation of Na^+/H^+ exchange isoform 1 mRNA expression in isolated rat hearts. Am J Physiol 1999; 277:H993–8.

[85] Sandmann S, Yu M, Kaschina E, Blume A, Bouzinova E, Aalkjaer C, et al. Differential effects of angiotensin AT1 and AT2 receptors on the expression, translation and function of the Na^+–H^+ exchanger and Na^+–HCO_3^- symporter in the rat heart after myocardial infarction. J Am Coll Cardiol 2001;37:2154–65.

[86] Camilion de Hurtado MC, Portiansky EL, Perez NG, Rebolledo OR, Cingolani HE. Regression of cardiomyocyte hypertrophy in SHR following chronic inhibition of the Na(+)/H(+) exchanger. Cardiovasc Res 2002;53:862–8.

[87] Chen L, Gan XT, Haist JV, Feng Q, Lu X, Chakrabarti S, et al. Attenuation of compensatory right ventricular hypertrophy and heart failure following monocrotaline-induced pulmonary vascular injury by the Na^+–H^+ exchange inhibitor cariporide. J Pharmacol Exp Ther 2001; 298:469–76.

[88] Cingolani HE, Rebolledo OR, Portiansky EL, Perez NG, Camilion de Hurtado MC. Regression of hypertensive myocardial fibrosis by Na(+)/H(+) exchange inhibition. Hypertension 2003;41:373–7.

[89] Ennis IL, Escudero EM, Console GM, Camihort G, Dumm CG, Seidler RW, et al. Regression of isoproterenol-induced cardiac hypertrophy by Na^+/H^+ exchanger inhibition. Hypertension 2003;41:1324–9.

[90] Karmazyn M. Role of sodium–hydrogen exchange in cardiac hypertrophy and heart failure: a novel and promising therapeutic target. Basic Res Cardiol 2001;96:325–8.

[91] Karmazyn M, Liu Q, Gan XT, Brix BJ, Fliegel L. Aldosterone increases NHE-1 expression and induces NHE-1-dependent hypertrophy in neonatal rat ventricular myocytes. Hypertension 2003;42:1171–6.

[92] Karmazyn M. Role of NHE-1 in cardiac hypertrophy and heart failure. p. 211–219. In: Karmazyn M, Avkiran M, Fliegel L, editors. The Na^+/H^+ Exchanger, From Molecular to Its Role in Disease. Boston: Kluwer Academic Publishers; 2003. 318 pp.

[93] Cingolani HE, Alvarez BV, Ennis IL, Camilion de Hurtado MC. Stretch-induced alkalinization of feline papillary muscle: an autocrine-paracrine system. Circ Res 1998;83:775–80.

[94] Perez NG, Alvarez BV, Camilion de Hurtado MC, Cingolani HE. pHi regulation in myocardium of the spontaneously hypertensive rat. Compensated enhanced activity of the Na(+)–H+ exchanger. Circ Res 1995;77:1192–200.

[95] Cingolani HE, Ennis IL. Sodium–hydrogen exchanger, cardiac overload, and myocardial hypertrophy. Circulation 2007;115:1090–100.

[96] Dulce RA, Hurtado C, Ennis IL, Garciarena CD, Alvarez MC, Caldiz C, et al. Endothelin-1 induced hypertrophic effect in neonatal rat cardiomyocytes: involvement of Na^+/H^+ and Na^+/Ca^{2+} exchangers. J Mol Cell Cardiol 2006;41:807–15.

[97] Takimoto E, Yao A, Toko H, Takano H, Shimoyama M, Sonoda M, et al. Sodium calcium exchanger plays a key role in alteration of cardiac function in response to pressure overload. FASEB J 2002;16:373–8.

[98] Hayasaki-Kajiwara Y, Kitano Y, Iwasaki T, Shimamura T, Naya N, Iwaki K, et al. Na(+) influx via Na(+)/H(+) exchange activates protein kinase C isozymes delta and epsilon in cultured neonatal rat cardiac myocytes. J Mol Cell Cardiol 1999;31:1559–72.

[99] Rugale C, Delbosc S, Cristol JP, Mimran A, Jover B. Sodium restriction prevents cardiac hypertrophy and oxidative stress in angiotensin II hypertension. Am J Physiol Heart Circ Physiol 2003;284: H1744–50.

[100] Javadov S, Baetz D, Rajapurohitam V, Zeidan A, Kirshenbaum LA, Karmazyn M. Antihypertrophic effect of Na^+/H^+ exchanger isoform 1 inhibition is mediated by reduced mitogen-activated protein kinase activation secondary to improved mitochondrial integrity and decreased generation of mitochondrial-derived reactive oxygen species. J Pharmacol Exp Ther 2006;317:1036–43.

[101] Ennis IL, Garciarena CD, Escudero EM, Perez NG, Dulce RA, Camilion de Hurtado MC, et al. Normalization of the calcineurin pathway underlies the regression of hypertensive hypertrophy induced by Na^+/H^+ exchanger-1 (NHE-1) inhibition. Can J Physiol Pharmacol 2007;85: 301–10.

[102] Engelhardt S, Hein L, Keller U, Klambt K, Lohse MJ. Inhibition of Na(+)–H(+) exchange prevents hypertrophy, fibrosis, and heart failure in beta(1)-adrenergic receptor transgenic mice. Circ Res 2002;90: 814–9.

[103] Jandeleit-Dahm K, Hannan KM, Farrelly CA, Allen TJ, Rumble JR, Gilbert RE, et al. Diabetes-induced vascular hypertrophy is accompanied by activation of Na(+)–H(+) exchange and prevented by Na(+)–H(+) exchange inhibition. Circ Res 2000;87:1133–40.

[104] Kinugawa K, Minobe WA, Wood WM, Ridgway EC, Baxter JD, Ribeiro RC, et al. Signaling pathways responsible for fetal gene induction in the failing human heart: evidence for altered thyroid hormone receptor gene expression. Circulation 2001;103:1089–94.

[105] Izumo S, Nadal-Ginard B, Mahdavi V. Protooncogene induction and reprogramming of cardiac gene expression produced by pressure overload. Proc Natl Acad Sci U S A 1988;85:339–43.

[106] Rieder CV, Fliegel L. Developmental regulation of Na(+)/H(+) exchanger expression in fetal and neonatal mice. Am J Physiol 2002;283: H273–83.

[107] Rieder CV, Fliegel L. Transcriptional regulation of Na^+/H^+ exchanger expression in the intact mouse. Mol Cell Biochem 2003;243:87–95.

[108] Haworth RS, Yasutake M, Brooks G, Avkiran M. Cardiac Na^+/H^+ exchanger during post-natal development in the rat: changes in mRNA expression and sarcolemmal activity. J Mol Cell Cardiol 1997;29: 321–32.

[109] Chen F, Jarmakani JM, Van Dop C. Developmental changes in mRNA encoding cardiac Na^+/H^+ exchanger (NHE-1) in rabbit. Biochem Biophys Res Commun 1995;212:960–7.

[110] Miller RT, Counillon L, Pages G, Lifton RP, Sardet C, Pouyssegur J. Structure of the 5′-flanking regulatory region and gene for the human growth factor-activatable Na^+/H^+ exchanger NHE-1. J Biol Chem 1991; 266:10813–9.

[111] Blaurock NC, Reboucas NA, Kusnezov JL, Igarashi P. Phylogenetically conserved sequences in the promoter of the rabbit sodium–hydrogen exchanger isoform 1 gene (NHE1 SLC9A1). Biochim Biophys Acta 1995;1262:159–63.

[112] Facanha AL, dos Reis MC, Montero-Lomeli M. Structural study of the porcine Na^+/H^+ exchanger NHE1 gene and its 5′- flanking region. Mol Cell Biochem 2000;210:91–9.

[113] Dyck JRB, Silva NLCL, Fliegel L. Activation of the Na^+/H^+ exchanger gene by the transcription factor AP-2. J Biol Chem 1995;270:1375–81.

[114] Wang H, Singh D, Yang W, Dyck JRB, Fliegel L. Structure and analysis of the mouse Na^+/H^+ exchanger (NHE1) gene: homology and conservation of splice sites. Mol Cell Biochem 1996;165:155–9.

[115] Horie S, Moe O, Yamaji Y, Cano A, Miller RT, Alpern RJ. Role of protein kinase C and transcription factor AP-1 in the acid-induced increase in Na/H antiporter activity. Proc Natl Acad Sci U S A 1992;89: 5236–40.

[116] Kolyada AY, Johns CA, Madias NE. Role of C/EBP proteins in hepatic and vascular smooth muscle transcription of human NHE1 gene. Am J Physiol 1995;269:C1408–16.

[117] Kolyada AY, Lebedeva TV, Johns CA, Madias NE. Proximal regulatory elements and nuclear activities required for transcription of the human Na^+/H^+ exchanger (NHE-1) gene. Biochim Biophys Acta 1994;1217: 54–64.

[118] Yang W, Dyck JRB, Fliegel L. Regulation of NHE1 expression in L6 muscle cells. Biochim Biophys Acta 1996;1306:107–13.

[119] Dyck JRB, Fliegel L. Specific activation of the Na^+/H^+ exchanger during neuronal differentiation of embryonal carcinoma cells. J Biol Chem 1995;270:10420–7.

[120] Yang W, Wang H, Fliegel L. Regulation of Na^+/H^+ exchanger gene expression. Role of a novel poly(dA:dT) element in regulation of the NHE1 promoter. J Biol Chem 1996;271:20444–9.

[121] Fernandez-Rachubinski F, Fliegel L. COUP-TF I and COUP-TFII regulate expression of the NHE through a nuclear hormone responsive element with enhancer activity. Eur J Biochem 2001;268:620–34.

[122] Li X, Misik AJ, Rieder CV, Solaro RJ, Lowen A, Fliegel L. Thyroid hormone receptor alpha 1 regulates expression of the Na^+/H^+ exchanger (NHE1). J Biol Chem 2002;277:28656–62.

Author's personal copy

[123] Yang W, Dyck JRB, Wang H, Fliegel L. Regulation of the NHE-1 promoter in the mammalian myocardium. Am J Physiol 1996;270:H259–66.

[124] Wang H, Yang W, Fliegel L. Identification of an HMG-like protein involved in regulation of Na^+/H^+ exchanger expression. Mol Cell Biochem 1997;176:99–106.

[125] Zhang GX, Kimura S, Nishiyama A, Shokoji T, Rahman M, Yao L, et al. Cardiac oxidative stress in acute and chronic isoproterenol-infused rats. Cardiovasc Res 2005;65:230–8.

[126] Laskowski A, Woodman OL, Cao AH, Drummond GR, Marshall T, Kaye DM, et al. Antioxidant actions contribute to the antihypertrophic effects of atrial natriuretic peptide in neonatal rat cardiomyocytes. Cardiovasc Res 2006;72:112–23.

[127] Lund AK, Peterson SL, Timmins GS, Walker MK. Endothelin-1-mediated increase in reactive oxygen species and NADPH Oxidase activity in hearts of aryl hydrocarbon receptor (AhR) null mice. Toxicol Sci 2005;88:265–73.

[128] Cheng TH, Shih NL, Chen CH, Lin H, Liu JC, Chao HH, et al. Role of mitogen-activated protein kinase pathway in reactive oxygen species-mediated endothelin-1-induced beta-myosin heavy chain gene expression and cardiomyocyte hypertrophy. J Biomed Sci 2005;12: 123–33.

[129] Akram S, Teong HF, Fliegel L, Pervaiz S, Clement MV. Reactive oxygen species-mediated regulation of the Na^+–H^+ exchanger 1 gene expression connects intracellular redox status with cells' sensitivity to death triggers. Cell Death Differ 2006;13:628–41.

[130] Kumar AP, Chang MK, Fliegel L, Pervaiz S, Clement MV. Oxidative repression of NHE1 gene expression involves iron-mediated caspase activity. Cell Death Differ 2007;14:1733–46.

[131] Spitzer KW, Vaughan-Jones RD. Regulation of intracellular pH in mammalian cells. Fig. 4, p 8, in The Na^+/H^+ Exchanger, p. 1–15. In: Karmazyn M, Avkiran M, Fliegel L, editors. From Molecular to Its Role in Disease. Boston: Kluwer academic Publishers; 2003. 318 pp.