

**Review**

# Physiological Functions and Regulation of the Na<sup>+</sup>/H<sup>+</sup> Exchanger [NHE1] in Renal Tubule Epithelial Cells

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## Key Words

NHE1 • Programmed cell death • Renal epithelial cells • Intracellular pH • Signaling pathways • Mechanical stretch

## Abstract

The sodium–hydrogen exchanger isoform-1 [NHE1] is a ubiquitously expressed plasma membrane protein that plays a central role in intracellular pH and cell volume homeostasis by catalyzing an electroneutral exchange of extracellular sodium and intracellular hydrogen. Outside of this important physiological function, the NHE1 cytosolic tail domain acts as a molecular scaffold regulating cell survival and actin cytoskeleton organization through NHE1-dependent signaling proteins. NHE1 plays main roles in response to physiological stress conditions which in addition to cell shrinkage and acidification, include hypoxia and mechanical stimuli, such as cell stretch. NHE1-mediated modulation of programmed cell death results from the exchanger-mediated changes in pHi, cell volume, and/or [Na<sup>+</sup>]<sub>i</sub>; and, it has recently become known that regulation of cellular signaling pathways are involved as well. This review focuses on NHE1 functions and regulations. We describe evidence showing how these structural actions integrate with ion translocation in regulating renal tubule epithelial cell survival.

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## Introduction

Maintenance of the intracellular ionic environment is necessary for homeostasis in living cells. The regulation of ion concentrations in cytoplasm is achieved through the balancing activities of ion pumps and transporters that are localized in the plasma membranes [1]. The carrier-mediated exchange of sodium for protons across biological membranes has

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been demonstrated in organisms from simple prokaryotes to more complex eukaryotes of the plant, fungi and animal kingdoms. This cation flux, conducted by a family of polytopic membrane proteins called Na<sup>+</sup>/H<sup>+</sup> antiporters or exchangers, are classified as secondary active transporters [2, 3]. The driving force for the exchange is not coupled directly to the hydrolysis of ATP, but is derived from the electrochemical gradient established for one of the solutes that drives the counter transport of the other [3].

The family of Na<sup>+</sup>/H<sup>+</sup> exchangers [NHEs], exhibits different tissue and cellular localization patterns and mediates a wide variety of physiological functions. Ten isoforms [NHE1–NHE10] have been identified within the mammalian NHE family [1, 3, 4]. The isoforms share approx 25%–70% amino acid identity, with calculated relative molecular masses ranging from approx. 74000 to 93000 Daltons [3, 5]. Hydropathy analysis of the exchanger predicts that they have similar membrane topologies with an N-terminal membrane domain consisting of 12 transmembrane [TM] segments followed by a hydrophilic C terminus that is oriented towards the cytoplasm [4].

The biochemical analyses of plasmalemmal NHEs suggest that they assemble and function as homodimers [6, 7], a structure that is similar to the recently discovered crystal structure of the bacterial *Escherichia coli* Na<sup>+</sup>/H<sup>+</sup> antiporter NhaA. However, unlike NhaA that operates as AN electrogenic transporter, the mammalian NHEs are electroneutral antiporters that exchange 1 H<sup>+</sup> per 1 Na<sup>+</sup> [8].

From the ten isoforms cloned, five different plasmalemmal-type NHEs, NHE1-5 have been identified. The NHE1 isoform is the 'housekeeping' isoform of the exchanger, and it is ubiquitously expressed in the plasma membrane of virtually all tissues. Although NHE2-NHE5 isoforms are also localized to the plasma membrane, they have a limited tissue distribution.

NHE2 is expressed in the apical membrane of the medullary thick ascending limb [MTAL] cells and in the apical membrane of intestinal epithelial cells [9, 10]. NHE3 is expressed at high levels in kidney apical membranes from proximal tubule [PT] and from TAL cells. Significant levels of NHE3 are also present in colon and small intestine cells [11, 12]. NHE4 is most abundant in the stomach, followed by intermediate levels in the small intestine and the colon and lesser amounts in renal TAL cells, in the brain, uterus and in skeletal muscles [10, 13].

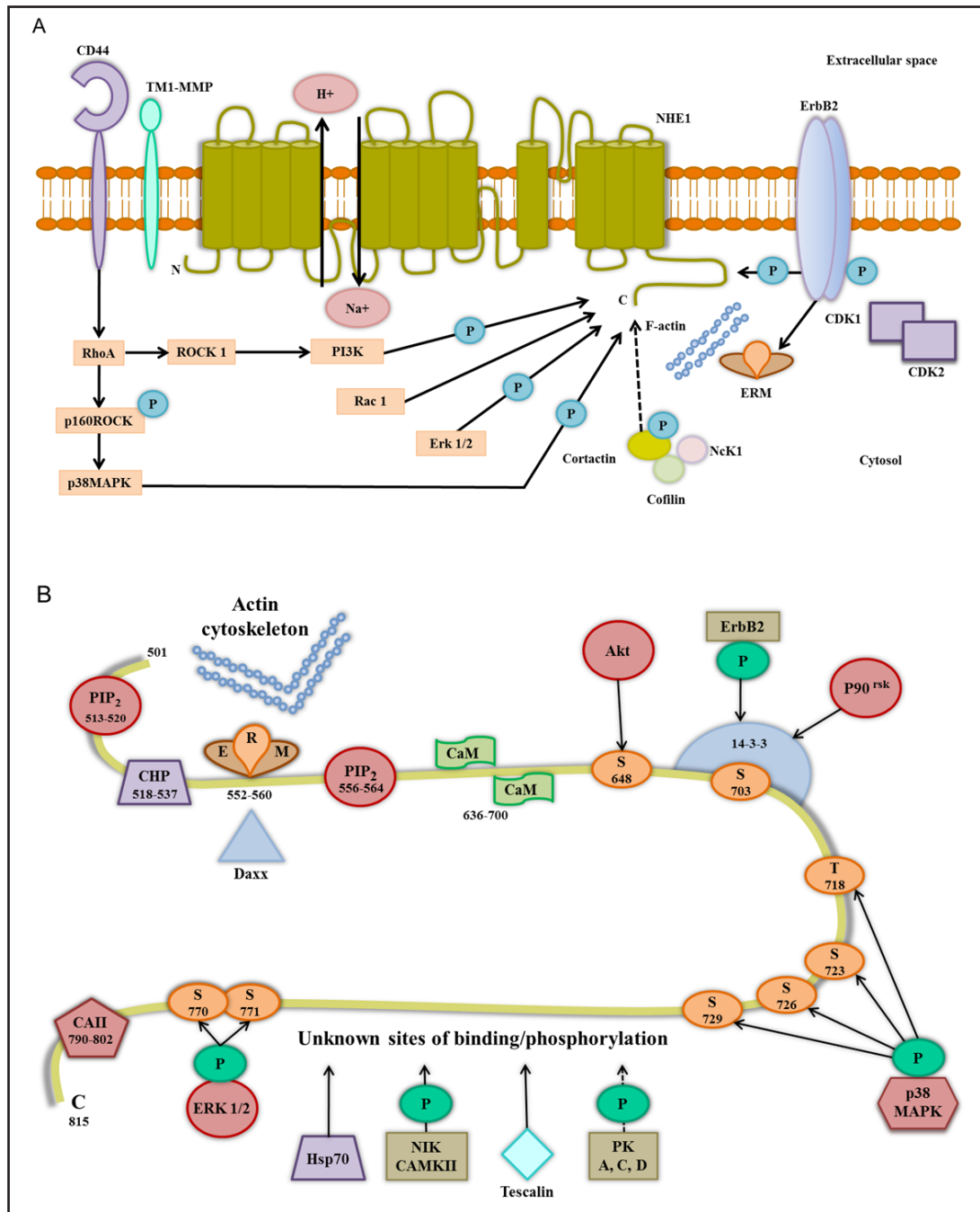
NHE5 has been shown to be expressed in the brain. It may also be localized at low levels in other non-epithelial tissues, as in the spleen, testis and skeletal muscles [14, 15]. NHE6-NHE9, ubiquitously expressed and localized in intracellular compartments are involved in the regulation of luminal pH and the cation concentration of the intracellular compartments [1, 16]. NHE6 is an endosomal Na<sup>+</sup>/H<sup>+</sup> exchanger that may regulate intravesicular pH and cell volume contributing to lysosomal biogenesis [17]. The NHE7 isoform is found predominantly in the trans-Golgi network and differs from the other NHE isoforms because of the influx of either Na<sup>+</sup> or K<sup>+</sup> in exchange for H<sup>+</sup> [17]. The transcript of NHE8 is relatively high in skeletal muscles and the kidney where it is abundant in proximal tubules in the outer medulla and cortex of the kidney as is reported by Goyal et al. [18]. NHE9 isoform is localized to late recycling endosomes [1, 4]. The recently identified NHE10 is expressed in osteoclasts. The exchanger is necessary for the differentiation and survival of these cells [19].

The present review will focus only on the functionality, regulation, and the role of the ubiquitous plasma membrane NHE isoform, NHE1. We describe evidence concerning the non-transport functions of the exchanger by analysing how structural functions join together with ion translocation to regulate renal epithelial cell survival.

### Structural and functional analysis of the Na<sup>+</sup>/H<sup>+</sup> exchanger

The NHE1 isoform is the most studied of the NHE family. The NHE1 gene [SLC9A1] maps to chromosome 1p36.11 in humans. Human NHE1 is an 815-amino acid glycoprotein with residues between 1–500, which contains three consensus N-glycosylation sites, and which

are all within the membrane domain [NH-terminal domain]. Residues 501–815 comprise the cytoplasmic tail [20] [Figure 1A and B]. The NH-terminal domain, which is known as the ion



**Fig. 1.** Diagram summarizing NHE1 regulation by proteins, cofactors, and protein kinases . A- NHE1 protein in the plasma membrane with regulatory pathways. B- The cytosolic regulatory tail of NHE1 is enlarged and indicates the location of regulatory binding sites and sites of phosphorylation. S: Serine residues. T: Threonine residues. CaM,calmodulin; CHP, calcineurin homologous protein; ERM, ezrin, radixin, moesin; PIP<sub>2</sub>,phosphatidylinositol4,5-biphosphate. 14-3-3-adaptor protein. This figure has been adapted by permission from the American Association for Cancer Research: Amith SR, Fliegel L: Regulation of the Na<sup>+</sup>/H<sup>+</sup> exchanger [NHE1] in breast cancer metastasis. Cancer Research, February 15, 2013, vol. 73 (4), pages 1259-1264, DOI: 10.1158/0008-5472.CAN-12-4031, [22].

translocation domain extrudes 1 intracellular proton in exchange for 1 extracellular sodium ion, protecting the cell from acidification and regulating intracellular pH (pHi) [21]. This is followed by hydrophilic, carboxyl-terminal cytosolic domain that regulates the membrane domain, and much of the regulation is by phosphorylation occurring in the distal 178 amino acids of the cytosolic domain.

Intracellular acidosis is the major stimulus involved in the regulation of NHE1 activity. NHE1 is essentially silent in most cell types at normal pHi, although it is quickly activated due to the decrease in pHi [22, 23]. Studies have proposed that this regulation occurs because of the interaction of H<sup>+</sup> with an allosteric modifier site ("pHi sensor") within the transport [24, 25].

NHE1 is known to require cellular ATP. The depletion of ATP, induced by treatment with metabolic inhibitors, abrogates the exchanger activity [26] even though there is no requirement for any energy input from ATP hydrolysis. As NHE1 is partially phosphorylated under basal conditions [27], it is possible that the NHE1 requirement for ATP is linked to the phosphorylation of the exchanger. However, some studies concluded that phosphorylation of NHE1 may not be involved in any ATP-dependent activation of NHE1 because ATP depletion does not change the phosphorylation state [28, 29]. A possible mechanism is that NHE1 may be directly modulated by ATP [30]. One investigation by Shimada-Shimizu et al shows that ATP directly binds to the proximal cytoplasmic region (Gly542-Pro598), which is critical for the ATP-dependent regulation of NHE1 [31]. These findings suggest that NHE1 is an ATP-binding transporter. In addition to the intracellular proton response, NHE1 activity is also regulated by the phosphorylation of key amino acids in its (300 amino acids) COOH<sup>-</sup> (C) terminal cytoplasmic domain and by interactions of the C-terminal tail with intracellular proteins and lipids [Fig. 1A and B].

Regulation of NHE1 at the molecular level has been studied in a variety of cell types. NHE1 regulators modify transport activity by altering the affinity for intracellular H<sup>+</sup>. These regulators of NHE1 are activated or controlled by extracellular growth factors, hormones and other agonists like Angiotensin II, serum, thrombin and the epidermal growth factor [22, 25].

### Physiological Functions of NHE1

NHE1 is often referred to as a housekeeping protein because one major function is to maintain intracellular pH and volume.

#### *NHE1 in the regulation of intracellular pH*

NHE1 activation results in electroneutral (1:1 stoichiometry) Na<sup>+</sup>/H<sup>+</sup> exchange. Intracellular acidification allosterically increases the activity of NHE which results in a rapid increase in pHi and because of this the exchanger protects cells from intracellular acidification. Mutant cell lines that are devoid of this exchange activity are extremely sensitive to acidosis [32, 33]. NHE1 also serves as a major Na<sup>+</sup> entry pathway in many cell types, and therefore regulates both sodium fluxes and cell volume after osmotic shrinkage [25, 34].

The volume- or osmolarity- sensitive site (s) within NHE1 exist between the NH2-terminus and residue 566. The mechanism by which NHE1 regulates cell volume remains elusive [35].

#### *NHE1 in the regulation of cell volume*

NHE1 often mediates the direct influx of the major extracellular cation sodium through the intracellular space along with the coupled subsequent osmotic movement of water. This process, known as regulatory volume increase [RVI], serves to counteract decreased cell volume.

Cell shrinkage results from the exposure to a hypertonic extracellular milieu. This event is then counterbalanced by the influx of sodium and chloride and the accompanying osmotic

driven influx of water which generates a compensatory cell swelling. Throughout the duration of the RVI, sodium is transported into the cell in exchange for protons mediated by NHE1 [36]. The net gain of sodium with negligible change in proton concentration results in a net osmotic gain due to the replacement of the extruded protons through a dissociation of cytosolic weak acids [37]. The entrance of water through water channels, such as aquaporin 1 [AQP1] comes after the influx of salt.

The intracellular concentration of bicarbonate also increases because the membrane is freely permeable to carbon dioxide as the pH rises. This growth in bicarbonate concentration drives chloride into the cell via electroneutral anion exchanger Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> (AE). This event then leads to a combined gain of sodium and chloride all the while extruding protons and bicarbonate, in sequence [38]. The translocated proton and bicarbonate recombine in the extracellular space to produce water and carbon dioxide. This CO<sub>2</sub> can re-enter and diffuse into the cell and is thus osmotically not relevant [38].

Considering this, NHE1 is the primary mechanism driving RVI in many cell types. This statement has been confirmed by deletion experiments. It has become clear that mutant cells lacking NHE1 fail to regain volume after imposed shrinkage [39].

In addition to its roles in regulating cellular pH and volume, NHE also initiates shifts in pHi that stimulate changes in the growth and functions of the cells [36, 40]. It is well established that activation of the Na-H exchanger NHE1 and increases in intracellular pH (pHi) are early responses to mitogens and have permissive effects in promoting cell proliferation [41]. In this regard, Putney et al showed that NHE1 activity and pHi regulate the timing of G<sub>2</sub>/M entry and transition and that efficient timing requires a pHi of greater than 7.30 which is promoted by NHE1 activity [42].

The exchanger also plays a role in either promoting or inhibiting programmed cell death, a function that changes depending on cell type [43, 44].

### **NHE1 regulation mediated by proteins, cofactors, protein kinases**

#### *Regulation by Phosphorylation*

The distal region of the C-terminal tail of NHE1, which it is comprised of aminoacids ~700–815, contains a number of serine and threonine residues. These are phosphorylated by protein kinases in response to sustained acidosis and /or to hormone and growth-factor stimulation [23, 27, 45].

The phosphorylation of residues in this region moves the set point of the exchanger by modifying transport activity. This happens because of the change in affinity for intracellular H<sup>+</sup>, which causes the exchange to be more active at greater alkaline pHi values. Several protein kinases involved in the regulation of NHE1 are known to phosphorylate specific amino acids within the cytosolic domain. Therefore, p160ROCK, a serine / threonine protein kinase, is a direct RhoA target, which mediates the Rho A-induced assembly of focal adhesions and stress fibers. p160 ROCK has been identified as an direct upstream activator of NHE1, which suggests that this kinase induces NHE1 activity and phosphorylation that are necessary for actin stress fiber assemblies [46]. Moreover, the activation of NHE1 is necessary for the RhoA-induced reorganization of the actin cytoskeleton [47]. This occurs as a consequence of the link between NHE1 and the actin binding proteins ezrin, radixin, and moesin [ERM] at residues 553–564 in the C-terminal tail of NHE1 [48].

Regulation of the cell cytoskeleton may be important for apoptosis [49] where cell adhesion may play a critical role [50]. We have previously shown RhoA pathway involvement through its translocation and its further membrane-associated GTP-bound activation in the regulation of apoptosis in 14 day obstructed cortex rat kidney epithelial cells [51].

#### *MAPKs and the regulation of membrane transport proteins*

NHE1 is the target of multiple signaling pathways, including those activated by tyrosine kinase receptors, G protein-coupled receptors and integrins. Several publications have

examined the role of mitogen-activated protein kinases [MAPKs] which include extracellular signal-regulated kinase [ERK], c-Jun N-terminal kinase [JNK] and p38 MAPK as potential mediators of NHE1 activation.

NHE1 is regulated by MAPKs under certain conditions, however, a number of studies have shown a central role for NHE1 upstream of MAPKs after a variety of stimuli [52].

MAPKs are important regulators of many membrane transport proteins at gene transcription level, via their downstream effectors [53]. Moreover, these signaling pathways and/or their downstream effectors also posttranscriptionally regulate the activity of multiple transporters. This happens either directly by phosphorylation of the transporter, or indirectly by phosphorylation of regulatory enzymes [54-56].

The direct involvement of phosphorylation in the regulation of NHE1 activity by MAPKs has been previously reported [57]. Takahashi E et al establish p90[*RSK*] as a serum-stimulated NHE-1 kinase and as a mediator of increased Na<sup>+</sup>/H<sup>+</sup> exchanges in vivo [58]. While studying how the hypertonicity activate ion transporters in *Xenopus oocytes*, Goss et al. found that the endogenous oocyte Na<sup>+</sup>/H<sup>+</sup> exchange activity [xoNHE] by hypertonic shrinkage required Cl<sup>-</sup>, and it was mediated by the upregulation of JNK [59].

Shrinkage-induced NHE1 activation is dependent on PKC and p38 MAPK, but not on ERK1/2. NHE function under both iso- and hypertonic conditions increases with the inhibition of serine/ threonine phosphatases, and this effect appears to be counteracted by the inhibition of PKC [60].

The protein kinase C [PKC] has been the most extensively investigated kinase, which is involved in this mechanism. The activation of PKC, either directly by phorbol esters [61] or indirectly via cell surface receptors [62], leads to an increase of the exchanger in a variety of cell types whereas putative PKC inhibitors block such effects [62, 63].

It has also been proposed that NHE1 is directly phosphorylated by p38 MAPK [64]. Alkalinization requires the stress p38 mitogen-activated protein kinase [MAPK]. In relation to this evidence, inhibition of p38 MAPK activity with pharmacological inhibitors or the expression of a dominant negative kinase prevents this alkalinization. Activated p38 MAPK is directly phosphorylated at the C terminus of NHE1 within a 40-aminoacid region.

A substantial body of studies indicate that NHE1 can regulate the activity of ERK1/2, JNK, and p38 MAPK. Notwithstanding, the effects of NHE1 on MAPK activity are highly contextual and cell type-specific.

Rentsch and al. have worked on fibroblasts demonstrating that ischemia anoxia is associated with an increased activity of NHE1 and also with p38 MAPK but at a slower rate. ERK 1/2 was not affected [65]. In Ehrlich Lettre Ascites cells (ELA cells) osmotic cell shrinkage upregulates NHE1 and negatively regulates ERK1/2 activity [66]. The effect of NHE1 on MAPK activity has also been observed in epithelial cells.

The rapid activation of NHE1 by Aldosterone at nanomolar concentrations, a non-genomic mechanism, involves ERK1/2. Consequently, in Madin-Darby canine kidney [MDCK] cells, Aldosterone modulates Na<sup>+</sup>/H<sup>+</sup> exchange by means of ERK1/2 [67]. Therefore we can say that in certain cell types, the MAPK cascade may represent an additional pathway by mediating the rapid Aldosterone effects.

It has been recently demonstrated that acute sustained intracellular acidosis [SIA] in MDCK cells occurs through an ERK-dependent pathway affecting the phosphorylation of the amino acids in the cytosolic regulatory tail of NHE. It is interesting to note that this was blocked by treatment with the MEK inhibitor U0126 [68].

While under several conditions, NHE1 is regulated by MAPKs, recent studies have, conversely, implicated NHE1 in the regulation of MAPK activity.

The precise pathways leading from NHE1 to MAPK regulation need to be determined. These studies should look at the roles of the transported ions in relation to those events involving the conformational coupling of NHE1 to enzymes or cytoskeletal elements [52, 69].

### *Regulatory proteins and cofactors*

Much of the hormonal regulation of NHE1 is not due just to phosphorylation, but is also mediated by several regulatory proteins and cofactors. These include calcium binding proteins that associate with the cytosolic regulatory tail. Calmodulin [CaM] likewise binds to a high- and low-affinity site which is located between amino acids 636 and 700 and blocks an auto-inhibitory site, thereby activating NHE1 as has been studied by Fliegel et al. [22]. The calcineurin homologous protein [CHP] group of regulatory calcium-binding proteins is also bound to the cytosolic regulatory tail. CHP1 and CHP2 bind to NHE1 in the amino acid regions of 518 to 537 [22] (Figure1B).

Recent results have demonstrated that CHP1 binding has two functions. First, it is important for antiporter activity; and, second, for the stabilization of NHE1 because of its ability to mask a degradation signal [70]. In conclusion, increased stability and the translocation of NHE1 is mediated by the direct binding of CHP1.

Furthermore, NHE1 has many different binding partners. This suggests that NHE1 without binding to CHP1 proteins may have an unstable conformation even if they can localize to the plasma membrane [71]. CHP2 is highly expressed in tumor cells; it is protective against serum deprivation-induced cell death by increasing pHi [71], and may play a role in the enhanced proliferation of tumor cells [72].

CHP-related protein tescalcin or CHIP3 [binding site uncertain] is thought to promote maturation and cell surface stability of the NHE1C-terminus of the Na<sup>+</sup>/H<sup>+</sup> exchanger. This protein exists primarily as a monomeric protein that binds regulatory tescalcin and can change conformation depending on pH and calcium [73].

The signaling molecule phosphatidylinositol 4,5-biphosphate [PIP2] binds to NHE1 at two putative PIP2-binding motifs within the C-terminal domain at residues 513–520 and 556–564 (Figure 1B). The binding of PIP2 to NHE1 is required for the optimal activity of the exchanger, and this interaction at least partially, accounts for the ATP dependence of NHE1. It has been demonstrated that PIP2 depletion leads to an ATP-dependent inhibition of NHE1 [74]. In addition, the actin-binding ERM [i.e., ezrin, radixin, and moesin] proteins bind to NHE1 between aminoacids 552 and 560, and they manage the proper localization of NHE1 to the plasma membrane as well as the maintenance of cell shape [75]. As has been demonstrated by Schelling et al., PI(4,5) P2 target NHE1 and ERM to the same plasma membrane domain. NHE1-ERM interaction leads to the formation of a signaling complex that includes phosphatidylinositol 3-kinase (PI3K) and, ultimately, the prosurvival kinase Akt. This complex phosphorylates multiple substrates, resulting in apoptosis inhibition in renal tubular epithelial cells [44].

Carbonic Anhydrase II [CAII], an enzyme that catalyzes the production of HCO<sub>3</sub><sup>-</sup> and H<sup>+</sup> from the hydration of CO<sub>2</sub>, binds to NHE1 at amino acid residues [790 to 802] increasing the activity of the exchanger [76].

The 14-3-3-adaptor protein only binds to NHE1 when it is phosphorylated at S703, which results in NHE1 activation that protects S703 from dephosphorylation. In this way, the functional binding of 14-3-3 to the exchanger is necessary for the activation of NHE1 by serum [77]. In addition, heat shock protein 70 [Hsp70] is linked to NHE1 and may play a role in the protein folding [78].

### *Na<sup>+</sup>/H<sup>+</sup> Exchangers in the Renal Regulation of Acid-Base Balance*

The kidney plays key roles in extracellular fluid pH homeostasis by reclaiming bicarbonate [HCO<sub>3</sub><sup>-</sup>] filtered at the glomerulus and generating the consumed HCO<sub>3</sub><sup>-</sup> by secreting protons [H<sup>+</sup>] into the urine. Intracellular [Na<sup>+</sup>] in renal epithelia is less than 20 mmol/L, whereas basolateral [Na<sup>+</sup>] ranges from 140 mmol/L in the renal cortex to as high as 300 mmol/L in the deep medulla [79, 80]. Newly synthesized NHE1 is localized in both apical and basolateral plasma membranes in polarized epithelial cells. However, mature NHE1 is localized almost exclusively in the basolateral membrane.

NHE1 has been shown to be expressed at the basolateral membrane of all nephron segments in the kidney, with the exception of the macula densa and cortical collecting duct intercalated cells [80, 81].

NHE3 has been observed in the apical surface in the proximal tubules as in the loop of Henle. Proximal tubule cells secrete H<sup>+</sup> from the cytosol across the microvillus apical membrane into the lumen via NHE3 and the vacuolar-type H<sup>+</sup>-ATPase [82].

The secreted H<sup>+</sup> and luminal HCO<sub>3</sub><sup>-</sup> is converted to CO<sub>2</sub> and H<sub>2</sub>O by carbonic anhydrase IV, an apical membrane-associated carbonic anhydrase [CA] [83]. The CO<sub>2</sub> and H<sub>2</sub>O rapidly reenter the cell across the apical membrane.

In the cytosol, CO<sub>2</sub> and H<sub>2</sub>O are converted back into HCO<sub>3</sub><sup>-</sup> and H<sup>+</sup> by CA II, the HCO<sub>3</sub><sup>-</sup> exiting with Na<sup>+</sup> across the basolateral membrane via the renal electrogenic Na<sup>+</sup>/HCO<sub>3</sub><sup>-</sup> cotransporter [NBCe1-A] at a stoichiometry of 3:1 into the interstitial space and ultimately in the blood [84].

Most of the H<sup>+</sup> secreted into the PT lumen titrates filtered HCO<sub>3</sub><sup>-</sup>, resulting in "HCO<sub>3</sub><sup>-</sup> reabsorption". The remainder of the secreted H<sup>+</sup> leads to the creation of "new HCO<sub>3</sub><sup>-</sup>" [85].

#### *The medullary thick ascending limb*

In the rat kidney, the thick ascending limb of the loop of Henle reabsorbs a significant amount of the filtered HCO<sub>3</sub><sup>-</sup>. The medullary thick ascending limb [MTAL] of the rat participates in the renal regulation of acid-base balance by reabsorbing much of the filtered HCO<sub>3</sub><sup>-</sup> that escapes the proximal tubule [86]. HCO<sub>3</sub><sup>-</sup> absorption in the MTAL is achieved as a two-step process involving; 1- the secretion of H<sup>+</sup> across the apical membrane mediated by the apical membrane Na<sup>+</sup>/H<sup>+</sup> exchanger NHE3 and 2- the transport of the HCO<sub>3</sub><sup>-</sup> across the basolateral membrane into the interstitium and then to the blood [87]. The mechanism of basolateral HCO<sub>3</sub><sup>-</sup> efflux in the MTAL involves a Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> exchange [88].

Good et al have demonstrated that basolateral membrane Na<sup>+</sup>/H<sup>+</sup> exchange enhances transepithelial HCO<sub>3</sub><sup>-</sup> absorption in the MTAL. The increase in HCO<sub>3</sub><sup>-</sup> absorption seems to be the result of basolateral membrane Na<sup>+</sup>/H<sup>+</sup> exchange effect to secondarily increase the activity of apical membrane Na<sup>+</sup>/H<sup>+</sup> exchange [89].

It has been demonstrated that basolateral NHE1 regulates apical NHE3 and HCO<sub>3</sub><sup>-</sup> absorption in the MTAL, by controlling the organization of the actin cytoskeleton [90]. In the renal medullary thick ascending limb [MTAL], inhibiting the basolateral NHE1 Na<sup>+</sup>/H<sup>+</sup> exchanger with amiloride or nerve growth factor [NGF] results secondarily in inhibition of the apical NHE3 Na<sup>+</sup>/H<sup>+</sup> exchanger, thereby decreasing transepithelial HCO<sub>3</sub><sup>-</sup> absorption [91]. Moreover in NHE1 knockout mice isolated microperfused thick ascending limbs [TALs], decreased HCO<sub>3</sub><sup>-</sup> absorption have been shown when compared with wild-type mice TALs [90].

These findings have been postulated as regulatory cross-talk between basolateral NHE1 and apical NHE3 because pharmacologic inhibition of basolateral NHE1 downregulates apical NHE3 activity [87].

In the TAL a significant amount of the luminal ammonium is reabsorbed, shunting the distal tubule, and it is secreted again in the collecting duct. NHE1 and/or NHE4 may play a role in the transepithelial transport of ammonium by mediating NH<sub>4</sub><sup>+</sup> extrusion at the basolateral membrane. Apical membrane NHE3 and NHE2 are responsible for the absorption of most of the remaining luminal bicarbonate following the same mechanism as in the proximal tubule [79, 85].

### **Involvement of NHE1 in epithelial renal cell survival**

NHE1, in addition to its effect on the regulation of cellular pH and volume, plays a role in either promoting or inhibiting programmed cell death depending on the cell type. The destruction of renal tubular cells [RTC] by apoptosis [92, 93] leads to tubular atrophy, a



hallmark of chronic renal diseases [94]. Significant epithelial cell apoptosis within the proximal tubule has been demonstrated in progressive renal disease [95].

After exposure to an injury stimulus, epithelium-derived cells are relatively resistant to apoptosis due to their great capacity to expand their intracellular volume through RVI which differs from neuronal cells and lymphocytes [96].

RVI is carried out by NHE1 activation, and depending on the cell type, by the AE2 isoform of the Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> exchanger and /or the Na<sup>+</sup>/K<sup>+</sup>/2Cl<sup>-</sup> symporter [97, 98].

If these RVI- dependent transporters are robustly activated after the initiation of an apoptotic stimulus, the restoration of intracellular volume may stop programmed cell death [99]. On the contrary, for a cell to undergo apoptosis, RVI must be overcome or inhibited [98, 99].

Neither AE2 nor the types 1 or 2 bumetanide-sensitive cotransporter [BSC-1 or BSC-2, respectively] isoforms of the Na<sup>+</sup>/K<sup>+</sup>/2Cl<sup>-</sup> symporter are expressed in proximal tubule cells [100, 101]. As long as NHE1 is ubiquitously expressed within the proximal tubule, the exchanger is critical for RTC survival by promoting resistance to apoptotic cell shrinkage.

In animal models of progressive renal disease, the proximal tubule segment triggers abundant cell apoptosis [92, 99]. This process is characterized by cell volume shrinkage and cytosol acidification leading to the activation of pro-apoptotic caspases [102] and conformational changes in Bax [103], which are consistent with a pH threshold for triggering cell death pathways [92, 99].

Schelling et al investigated whether RTC apoptosis requires caspase cleavage of NHE1. Staurosporine and hypertonic NaCl-induced RTC cell death was associated with cell shrinkage and diminished cytosolic pH, and apoptosis was enhanced by amiloride analogs, suggesting NHE1 activity opposes to cell death [104].

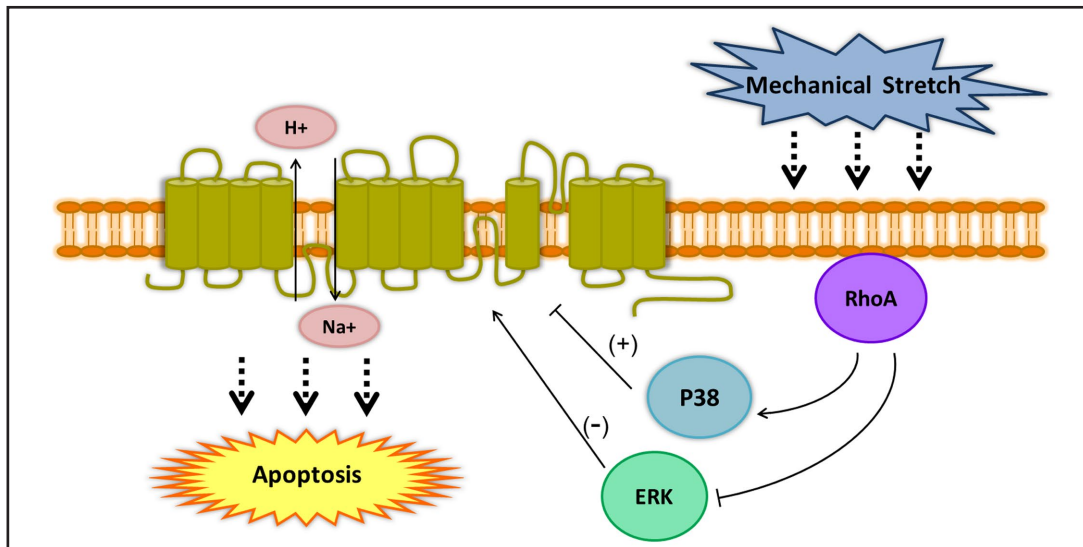
Under normal circumstances, the NHE1 “housekeeping” activity is not required. However, the exchanger plays an important role in the condition of cellular stress. Experimental studies have been performed on mice targeting NHE1 gene deletion or a spontaneous point mutation that introduces a premature stop codon. This results in a truncation between the 11th and 12th NHE1 transmembrane domains and the loss of NHE1 function (*Swemice*) [105]. *Swemice* treated with the podocyte toxin Adriamycin, developed increased cortical tubular epithelial cell apoptosis when compared to wild-type controls [104].

In addition, streptozotocin induced diabetes in NHE1-deficient *swe/swe* mice, which gives rise to greater azotemia, albuminuria and tubulointerstitial pathologies when compared to wild-type control animals. Increased RTC apoptosis was noted in these mice suggesting that the loss of NHE1 function leads to tubular atrophy, an indicator of kidney disease [106].

In the context of these findings, Schelling et al proposed that the combination of the loss of NHE1 function and glomerular injury from Adriamycin or Streptozotocin was sufficient for overcoming the protective genetic background effects [104, 106].

We demonstrated that tubular epithelial cell apoptosis is associated with decreased NHE1 expression in a neonatal rat model of ureteral obstruction [107]. After 14 days of ureteral obstruction, there were consistently more apoptotic cells in cortical ducts [CD] than in proximal tubules [PT], which is associated with a lower expression of NHE1 at the mRNA and protein levels. There was an increased expression of the Bax/Bcl2 ratio, which is linked to decreased pro-caspase-3 protein levels and with increased caspase-3 activation. We also showed that inhibiting NHE1 by increasing doses of EIPA induced epithelial cell apoptosis and increased caspase-3 activity in a dose-dependent manner in neonatal obstruction. Therefore, the decreased NHE1 expression could be a signal-transduction event participating in the induction of the apoptosis through the regulation of the Bcl-2 gene family and the activation of caspase-3 [107].

In another study, we found that the convergence of two independent signal pathways, the RhoA GTPase and pERK along with the concurrent inhibition of JNK MAP kinase were required for the apoptotic response in 14 day kidney cortex obstructed tubular epithelial cells [51].



**Fig. 2.** A schematic diagram of the mechanisms involved in NHE1-mediated regulation of epithelial cell apoptosis in response to cyclic stretch. In human proximal tubule cells RhoA signaling pathway activation after mechanical stretch induces ERK1/2 inhibition and p38 activation, which downregulates NHE1 expression and activity and finally triggers caspase 3 activation and apoptosis induction.

In recent studies, we reproduced mechanical proximal tubule cell deformation by exposing HK-2 cells to cyclic mechanical stretch. In a time-dependent manner, as mechanical deformation increased, NHE1 protein expression gradually decreased reaching the maximum decrease at 180 min of cyclic stretch. This is in accordance with the highest activation of caspase 3 and apoptosis induction.

By silencing the exchanger by NHE1-siRNA, we further confirmed the direct participation of NHE1 in cell survival due to a decreased Bcl2 and increased caspase 3 protein expressions. Moreover, after NHE1 knockdown and subsequent mechanical deformation in HK-2; apoptotic cells exceeded the values observed in non-targeted NHE1 stretched cells as well as those values recorded from EIPA treated stretched cells [108].

NHE1 is dependent on protein scaffolding even though it functions as a pHi regulator. We showed an early and progressive activation of RhoA in HK-2 cells exposed to cyclic stretch. Higher membrane bound RhoA precedes NHE1 downregulation and reaches its maximal activation state at 180 minutes during mechanical deformation, in agreement to NHE1 downregulation and apoptosis induction [108].

In HK-2 stretched cells, early ERK1/2 activation with an intensive phosphorylation demonstrates a later decrease of ERK1/2 at 180 min of cyclic stretch. Contrarily, p38 MAPKinase reaches its maximal phosphorylation state at 180 min of mechanical stretch [108]. We observed an increase in NHE1 protein expression without caspase 3 activation after p38 inhibition. On the other hand, ERK 1/2 inhibition leads to decreased NHE1 expression and caspase 3 activation.

The manipulation of MAPKs activation also affects NHE1 function, showing diminished pHi recovery after ERK 1/2 inhibition and NHE1 activity similar to control when HK2 cells were incubated with the p38 inhibitor.

Taken together, our data obtained from human proximal tubule cells suggest that RhoA signaling pathway activation following mechanical stretch induces ERK 1/2 inhibition and p38 activation. This leads to the downregulation of NHE1 expression and activity, which consequently triggers caspase 3 activation and apoptosis induction [108], see Fig 2.

Experiments in which NHE1-null proximal tubule cells demonstrate an enhanced sensitivity to multiple apoptotic stimuli when compared with wild-type cells is evidence for a cytoprotective role of the exchanger [109].

## Conclusion

These data indicate that apoptotic stress triggers early NHE1-dependent defence mechanisms against cell volume reduction and intracellular acidification. However, this survival NHE1 effect can be overcome by overwhelmingly sustained apoptotic stimuli allowing programmed cell death.

## Disclosure Statement

All the authors declared no competing interests.

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## References

- 1 Nakamura N, Tanaka S, Teko Y, Mitsui K, Kanazawa H: Four Na<sup>+</sup>/H<sup>+</sup> exchanger isoforms are distributed to Golgi and post-Golgi compartments and are involved in organelle pH regulation. *J Biol Chem* 2005;280:1561-1572.
- 2 Noel J, Pouyssegur J: Hormonal regulation, pharmacology, and membrane sorting of vertebrate Na<sup>+</sup>/H<sup>+</sup> exchanger isoforms. *Am J Physiol Cell Physiol* 1995;268:C283-C296.
- 3 Orłowski J, Grinstein S: Diversity of the mammalian sodium/proton exchanger SLC9 gene family. *Pflügers Arch* 2004;447:549-565.
- 4 Slepko ER, Jan K, Rainey JK, Sykes BD, Fliegel L: Structural and functional analysis of the Na<sup>+</sup>/H<sup>+</sup> exchanger. *Biochem J* 2007;401:623-633.
- 5 Khadilkar A, Iannuzzi P, Orłowski J: Identification of sites in the second exo-membrane loop and ninth transmembrane helix of the mammalian Na<sup>+</sup>/H<sup>+</sup> exchanger important for drug recognition and cation translocation. *J Biol Chem* 2001;276:43792-43800.
- 6 Lacroix J, Poet M, Maehrel C, Counillon L: A mechanism for the activation of the Na<sup>+</sup>/H<sup>+</sup> exchanger NHE1 by cytoplasmic acidification and mitogens. *EMBO Rep* 2004;5:91-96.
- 7 Hisamitsu T, Ammar YB, Nakamura TY, Wakabayashi S: Dimerization is crucial for the function of the Na<sup>+</sup>/H<sup>+</sup> exchanger NHE1. *Biochemistry* 2006;45:13346-13355.
- 8 Hunte C, Screpanti E, Venturi M, Rimon A, Padan E, Michel H: Structure of a Na<sup>+</sup>/H<sup>+</sup> antiporter and insights into mechanism of action and regulation by pH. *Nature* 2005;435:1197-1202.
- 9 Orłowski J, Kandasamy RA, Shull GE: Molecular cloning of putative members of the Na<sup>+</sup>/H<sup>+</sup> exchanger gene family. *J Biol Chem* 1992;267:9331-9339.
- 10 Sun AM, Liu Y, Dworkin LD, Tse CM, Donowitz M, Yip KP: Na<sup>+</sup>/H<sup>+</sup> exchanger isoform 2 [NHE2] is expressed in the apical membrane of the medullary thick ascending limb. *J Membrane Biol* 1997;160:85-90.
- 11 Amemiya M, Loffing J, Lotscher M, Kaissling B, Apert RJ, Moe OW: Expression of NHE3 in the apical membrane of rat renal proximal tubule and thick ascending limb. *Kidney Int* 1995;48:1206-1215.
- 12 Noel J, Rou D, Pouyssegur J: Differential localization of Na<sup>+</sup>/H<sup>+</sup> exchanger isoforms [NHE1 and NHE3] in polarized epithelial cell lines. *J Cell Sci* 1996;109:929-939.
- 13 Chambrey R, St John PL, Eladari D, Quentin F, Warnock DG, Abrahamson DR, Podevin RA, Paillard M: Localization and functional characterization of Na<sup>+</sup>/H<sup>+</sup> exchanger isoform NHE4 in rat thick ascending limbs. *Am J Physiol Renal Physiol* 2001; 281: F707-F717.
- 14 Baird NR, Orłowski J, Szabo EZ, Zaun HC, Schultheis PJ, Menon AG: Molecular cloning, genomic organization, and functional expression of Na<sup>+</sup>/H<sup>+</sup> exchanger isoform 5 [NHE5] from human brain. *J Biol Chem* 1999;274:4377-4382.
- 15 Attaphitaya S, Park K, Melvin JE: Molecular cloning and functional expression of a rat Na<sup>+</sup>/H<sup>+</sup> exchanger

- [NHE5] highly expressed in brain. *J Biol Chem* 1999;274:4383-4388.
- 16 Numata M, Orlowski J: Molecular cloning and characterization of a novel [Na<sup>+</sup>, K<sup>+</sup>/H<sup>+</sup>] exchanger localized to the trans-Golgi network. *J Biol Chem* 2001;276:17387-17394.
  - 17 Brett CL, Wei Y, Donowitz M, Rao R: Human Na<sup>+</sup>/H<sup>+</sup> exchanger isoform 6 is found in recycling endosomes of cells, not in mitochondria. *Am J Physiol Cell Physiol* 2002;282:C1031-C1041.
  - 18 Goyal S, Vanden Heuvel G, Aronson PS: Renal expression of novel Na<sup>+</sup>/H<sup>+</sup> exchanger isoform NHE8. *Am J Physiol* 2003;284:F467-F473.
  - 19 Lee SH, Kim T, Park ES, Yang S, Jeong D, Choi Y, Rho J: NHE10, an osteoclast-specific member of the Na<sup>+</sup>/H<sup>+</sup> exchanger family, regulates osteoclast differentiation and survival. *Biochem Biophys Res Commun* 2008;369:320-326.
  - 20 Counillon L, Pouysstgurt J, Reithme RAF: The Na<sup>+</sup>/H<sup>+</sup> exchanger NHE-1 possesses N- and O-linked glycosylation restricted to the first N-terminal extracellular domain. *Biochemistry* 1994;33:10463-10469.
  - 21 Wakabayashi S, Fafournoux P, Sardet C, Pouyssegur J: The Na<sup>+</sup>/H<sup>+</sup> antiporter cytoplasmic domain mediates growth factor signals and controls "H<sup>+</sup>-sensing". *Proc Natl Acad Sci USA* 1992;89:2424-2428.
  - 22 Amith SR, Fliegel L: Regulation of the Na<sup>+</sup>/H<sup>+</sup> exchanger [NHE1] in breast cancer metastasis. *Cancer Res* 2013;73:1259-1264.
  - 23 Haworth RS, McCann C, Snabaitis AK, Roberts NA, Avkiran M: Stimulation of the plasma membrane Na<sup>+</sup>/H<sup>+</sup> exchanger NHE1 by sustained intracellular acidosis. Evidence for a novel mechanism mediated by the ERK pathway. *J Biol Chem* 2003;278:31676-31684.
  - 24 Putney LK, Denker SP, Barber DL: The changing face of the Na<sup>+</sup>/H<sup>+</sup> exchanger, NHE1: structure, regulation, and cellular actions. *Annu Rev Pharmacol Toxicol* 2002;42:527-552.
  - 25 Wakabayashi S, Sardet C, Fafournoux P, Counillon L, Meloche S, Pages G, Pouyssegur J: Structure function of the growth factor-activatable Na<sup>+</sup>/H<sup>+</sup> exchanger [NHE1]. *Rev Physiol Biochem Pharmacol* 1992;119:157-186.
  - 26 Kapus A, Grinstein S, Wasan S, Kandasamy R, Orlowski J: Functional characterization of three isoforms of the Na<sup>+</sup>/H<sup>+</sup> exchanger stably expressed in Chinese hamster ovary cells. ATP dependence, osmotic sensitivity, and role in cell proliferation. *J Biol Chem* 1994;269:23544-23552.
  - 27 Sardet C, Counillon L, Franchi A, Pouyssegur J: Growth factors induce phosphorylation of the Na<sup>+</sup>/H<sup>+</sup> antiporter, glycoprotein of 110 kD. *Science* 1990;247:723-726.
  - 28 Goss GG, Woodside M, Wakabayashi S, Pouyssegur J, Waddell T, Downey, GP, Grinstein S: ATP dependence of NHE-1, the ubiquitous isoform of the Na<sup>+</sup>/H<sup>+</sup> antiporter. Analysis of phosphorylation and subcellular localization. *J Biol Chem* 1994;269:8741-8748.
  - 29 Wakabayashi S, Bertrand B, Shigekawa M, Fafournoux P, Pouyssegur J: Growth factor activation and H<sup>+</sup>-sensing of the Na<sup>+</sup>/H<sup>+</sup> exchanger isoform 1 [NHE1]. Evidence for an additional mechanism not requiring direct phosphorylation. *J Biol Chem* 1994;269:5583-5588.
  - 30 Demarex N, Romanek RR, Orlowski J, Grinstein S: ATP dependence of Na<sup>+</sup>/H<sup>+</sup> exchange. Nucleotide specificity and assessment of the role of phospholipids. *J Gen Physiol* 1997;109:117-128.
  - 31 Shimada-Shimizu N, Hisamitsu T, Nakamura TY, Wakabayashi S: Evidence that Na<sup>+</sup>/H<sup>+</sup> exchanger 1 is an ATP-binding protein. *FEBS J* 2013;280:1430-1442.
  - 32 Grinstein S, Rotin D, Mason MJ: Na<sup>+</sup>/H<sup>+</sup> exchange and growth factor-induced cytosolic pH changes. Role in cellular proliferation. *Biochim Biophys Acta* 1989;988:73-97.
  - 33 Pouyssegur J, Sardet C, Franchi A, L'Allemain G, Paris S: A specific mutation abolishing Na<sup>+</sup>/H<sup>+</sup> antiport activity in hamster fibroblasts precludes growth at neutral and acidic pH. *Proc Natl Acad Sci USA* 1984;81:4833-4837.
  - 34 Rotin D, Grinstein S: Impaired cell volume regulation in Na<sup>+</sup>-H<sup>+</sup> exchange-deficient mutants. *Am J Physiol* 1989;257:C1158-C1165.
  - 35 Bianchini L, Kapus A, Lukacs G, Wasan S, Wakabayashi S, Pouyssegur J, Yu FH, Orlowski J, Grinstein S: Responsiveness of mutants of NHE1 isoform of Na<sup>+</sup>/H<sup>+</sup> antiport to osmotic stress. *Am J Physiol* 1995;269:C998-C1007.
  - 36 Alexander RT, Grinstein S: Na<sup>+</sup>/H<sup>+</sup> exchangers and the regulation of volume. *Acta Physiologica* 2006;187:159-167.
  - 37 Lang F, Busch GL, Ritter M, Völkl H, Waldegger S, Gulbins E, Häussinger D: Functional significance of cell volume regulatory mechanisms. *Physiol Rev* 1998;78:247-306.
  - 38 Grinstein S, Clarke CA, Rothstein A: Activation of Na<sup>+</sup>/H<sup>+</sup> exchange in lymphocytes by osmotically induced volume changes and by cytoplasmic acidification. *J Gen Physiol* 1983;82:619-638.

- 39 Grinstein S, Woodside M, Sardet C, Pouyssegur J, Rotin D: Activation of the Na<sup>+</sup>/H<sup>+</sup> antiporter during cell volume regulation. Evidence for a phosphorylation-independent mechanism. *J Biol Chem* 1992;25:23823-23828.
- 40 Hoffmann E, Simonsen LO: Membrane mechanisms in volume and pH regulation in vertebrate cells. *Physiol Rev* 1989;69:315-382.
- 41 Putney LK, Barber DL: Na<sup>+</sup>/H<sup>+</sup> exchange-dependent increase in intracellular pH times G2/M entry and transition. *J Biol Chem* 2003;278:44645-44649.
- 42 Wang J, Singh D, Fliegel L: The Na<sup>+</sup>/H<sup>+</sup> exchanger potentiates growth and retinoic acid induced differentiation of P19 embryonic carcinoma cells. *J Biol Chem* 1997;272:26545-26549.
- 43 Yang W, Dyck JRB, Fliegel L: Regulation of NHE1 expression in L6 muscle cells. *Biochim Biophys Acta* 1996;1306:107-113.
- 44 Wu KL, Khan S, Lakhe-Reddy S, Jarad G, Mukherjee A, Obejero-Paz C A, Konieczkowski M, Sedor JR, Schelling JR: The NHE1 Na<sup>+</sup>/H<sup>+</sup> exchanger recruits ezrin/radixin/moesin proteins to regulate Akt-dependent cell survival. *J Biol Chem* 2004;279:26280-26286.
- 45 Sardet C, Fafournoux P, Pouyssegur J: α-Thrombin, epidermal growth factor, and okadaic acid activate the Na<sup>+</sup>/H<sup>+</sup> exchanger, NHE1, by phosphorylating a set of common sites. *J Biol Chem* 1991;266:19166-19171.
- 46 Tominaga T, Ishizaki T, Narumiya S, Barber DL: p160ROCK mediates RhoA activation of Na<sup>+</sup>/H<sup>+</sup> exchange. *EMBO J* 1998;17:4712-4722.
- 47 Hooley R, Yu CY, Symons M, Barber DL: G alpha 13 stimulates Na<sup>+</sup>/H<sup>+</sup> exchange through distinct Cdc42-dependent and Rho-dependent pathways. *J Biol Chem* 1996;271:6152-6158.
- 48 Denker SP, Huang DC, Orłowski, J, Furthmayr H, Barber DL: Direct binding of the Na<sup>+</sup>/H<sup>+</sup> exchanger NHE1 to ERM proteins regulates the cortical cytoskeleton and cell shape independently of H<sup>+</sup> translocation. *Mol Cell* 2000;6:1425-1436.
- 49 Rouslahti E, Reed JC: Anchorage dependence, integrins and apoptosis. *Cell* 1994;77:477-478.
- 50 Nobes CD, Hall A: Rho, Rac, and CDC42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. *Cell* 1995;81:53-62.
- 51 Bocanegra V, Rinaldi Tosi M, Gil Lorenzo A, Cacciamani V, Manucha W, Fornés M, Vallés PG: Angiotensin AT1 receptor inhibition-induced apoptosis by RhoA GTPase activation and pERK1/2 signaling pathways in neonatal obstructive nephropathy. *Histol Histopathol* 2012;27:919-930.
- 52 Pedersen SF, Darborg BV, Rentsch ML, Rasmussen M: Regulation of mitogen-activated protein kinase pathways by the plasma membrane Na<sup>+</sup>/H<sup>+</sup> exchanger, NHE1. *Arch Biochem Biophys* 2007;462:195-201.
- 53 Sheikh-Hamad, Gustin MC: MAPkinases and the adaptive response to hypertonicity: functional preservation from yeast to mammals. *Am J Physiol Renal* 2004;287:F1102-F1110.
- 54 Klein JD, Lamitina ST, O'Neill WC: JNK is a volume-sensitive kinase that phosphorylates the Na-K-2Cl cotransporter in vitro. *Am J Physiol* 1999;277:C425-C431.
- 55 Hu Z, Wang Y, Graham WV, Su L, Musch MW, Turner JR: MAPKAPK-2 is a critical signaling intermediate in NHE3 activation following Na<sup>+</sup>-glucose cotransport. *J Biol Chem* 2006;281:24247-24253.
- 56 Schrader LA, Birnbaum SG, Nadin BM, Ren Y, Bui D, Anderson AE, Sweatt JD: ERK/MAPK regulates the Kv4.2 potassium channel by direct phosphorylation of the pore-forming subunit. *Am J Physiol Cell Physiol* 2006;290:C852-C861.
- 57 Bianchini L, L'Allemain G, Pouyssegur J: The p42/p44 mitogen-activated protein kinase cascade is determinant in mediating activation of the Na<sup>+</sup>/H<sup>+</sup> exchanger [NHE1 isoform] in response to growth factors. *J Biol Chem* 1997;272:271-279.
- 58 Takahashi E, Abe J, Gallis B, Aebersold R, Spring DJ, Krebs EG: p90 [RSK] is a serum-stimulates Na<sup>+</sup>/H<sup>+</sup> exchanger isoform-1 kinase. Regulatory phosphorylation of serine 703 of Na<sup>+</sup>/H<sup>+</sup> exchanger isoform-1. *J Biol Chem* 1999;274:20206-20214.
- 59 Goss GG, Jiang L, Vandorpe DH, Kieller D, Chernova MN, Robertson M, Alper SL: Role of JNK in hypertonic activation of Cl<sup>-</sup> dependent Na<sup>+</sup>/H<sup>+</sup> exchange in *Xenopus* oocytes. *Am J Physiol Cell Physiol* 2001;281:C1978-C1990.
- 60 Pederson SF, Varming C, Christensen ST, Hoffmann EK: Mechanisms of activation of NHE by cell shrinkage and by calyculin A in Ehrlich ascites tumor cells. *J Membr Biol* 2002;189:67-81.
- 61 Grinstein S, Goetz-Smith JD, Stewart D, Beresford B J, Mellors A: Protein phosphorylation during activation of Na<sup>+</sup>/H<sup>+</sup> exchange by phorbol esters and by osmotic shrinking. *J Biol Chem* 1986;261:8009-8016.
- 62 Wallert MA, Fröhlich O: α1-Adrenergic stimulation of Na<sup>+</sup>/H<sup>+</sup> exchange in cardiac myocytes. *Am J Physiol* 1992;263:C1096-C110.

- 63 Haworth RS, Sinnott-Smith J, Rozengurt E, Avkiran M: Protein kinase D inhibits plasma membrane Na<sup>+</sup>/H<sup>+</sup> exchanger activity. *Am J Physiol* 1999;277:C1202-C1209.
- 64 Khaled AR, Moor AN, Li A, Kim K, Ferris D K, Muegge K, Fisher RJ, Fliegel L, Durum SK: Trophic factor withdrawal: p38 mitogen-activated protein kinase activates NHE1, which induces intracellular alkalization. *Mol Cell Biol* 2001;21:7545-7557.
- 65 Rentsch ML, Ossum CG, Hoffmann EK, Pedersen SF: Roles of Na<sup>+</sup>/H<sup>+</sup> exchange in regulation of p38 mitogen-activated protein kinase activity and cell death after chemical anoxia in NIH3T3 fibroblasts. *Pflugers Arch* 2007;454:649-662.
- 66 Pedersen SF, Darborg BV, Rasmussen M, Nylandsted J, Hoffmann EK: The Na<sup>+</sup>/H<sup>+</sup> exchanger, NHE1, differentially regulates mitogen-activated protein kinase subfamilies after osmotic shrinkage in Ehrlich Lettre Ascites cells. *Cell Physiol Biochem* 2007;20:735-750.
- 67 Gekle M, Freudinger R, Mildenerberger S, Schenk K, Marschitz I, Schramek H: Rapid activation of Na<sup>+</sup>/H<sup>+</sup> exchange in MDCK-cells by aldosterone involves MAP-kinases ERK1/2. *Pflugers Arch* 2001;441:781-786.
- 68 Odunewu A, Fliegel L: Acidosis-mediated regulation of the NHE1 isoform of the Na<sup>+</sup>/H<sup>+</sup> exchanger in renal cells. *Am J Physiol Renal Physiol* 2013;305:F370-F381.
- 69 Levitan IB: Signaling protein complexes associated with neuronal ion channels. *Nat Neurosci* 2006;9:305-310.
- 70 Matsushita M, Tanaka H, Mitsui K, Kanazawa H: Dual functional significance of calcineurin homologous protein 1 binding to Na<sup>+</sup>/H<sup>+</sup> exchanger isoform1. *Am J Physiol* 2011;301:C280-C288.
- 71 Pang T, Wakabayashi S, Shigekawa M: Expression of calcineurin B homologous protein 2 protects serum deprivation-induced cell death by serum-independent activation of Na<sup>+</sup>/H<sup>+</sup> exchanger. *J Biol Chem* 2002;277:43771-43777.
- 72 Li QH, Wang LH, Lin YN, Chang GQ, Li HW, Jin WN, Hu RH, Pang TX: Nuclear accumulation of calcineurin B homologous protein 2 CHP2: results in enhanced proliferation of tumor cells. *Genes Cells* 2011;16:416-426.
- 73 Li X, Liu Y, Kay CM, Muller-Esterl W, Fliegel L: The Na<sup>+</sup>/H<sup>+</sup> exchanger cytoplasmic tail: Structure, function, and interactions with tescalcin. *Biochemistry* 2003;42:7448-7456.
- 74 Aharonovitz O, Zaun H C, Balla T, York J D, Orłowski J, Grinstein, S: Intracellular pH regulation by Na<sup>+</sup>/H<sup>+</sup> exchange requires phosphatidylinositol 4,5-bisphosphate. *J Cell Biol* 2000;150:213-224.
- 75 Baumgartner M, Patel H, Barber DL: Na<sup>+</sup>/H<sup>+</sup> exchanger NHE1 as plasma membrane scaffold in the assembly of signaling complexes. *Am J Physiol Cell Physiol* 2004;287:C844-C850.
- 76 Li X, Liu Y, Alvarez BV, Casey JR, Fliegel L: A novel carbonic anhydrase II binding site regulates NHE1 activity. *Biochemistry* 2006;45:2414-2424.
- 77 Lehoux S, Abe J, Florian JA, Berk BC: 14-3-3 Binding to Na<sup>+</sup>/H<sup>+</sup> exchanger isoform-1 is associated with serum-dependent activation of Na<sup>+</sup>/H<sup>+</sup> exchange. *J Biol Chem* 2001;276:15794-15800.
- 78 Silva NL, Haworth RS, Sing D, Fliegel L: The carboxyl-terminal region of the Na<sup>+</sup>/H<sup>+</sup> exchanger interacts with mammalian heat shock protein. *Biochemistry* 1995;34:10412-10420.
- 79 Bobulescu IA, Moe OW: Na<sup>+</sup>/H<sup>+</sup> exchangers in renal regulation of acid-base balance. *Semin Nephrol* 2006;26:334-344.
- 80 Biemesderfer D, Reilly RF, Exner M, Agarashi P, Aronson PS: Immunocytochemical characterization of Na<sup>+</sup>/H<sup>+</sup> exchanger isoform NHE1 in rabbit kidney. *Am J Physiol* 1992;263:F833-F840.
- 81 Peti-Peterdi J, Chambrey R, Bebok Z, Biemesderfer D, St John PL, Abrahamson DR, Warnock DG, Bell PD: Macula densa Na<sup>+</sup>/H<sup>+</sup> exchange activities mediated by apical NHE2 and basolateral NHE4 isoforms. *Am J Physiol* 2000;278:F452-F463.
- 82 Gluck SL, Underhill DM, Iyori M, Holliday LS, Kostrominova TY, Lee BS: Physiology and biochemistry of the kidney vacuolar H<sup>+</sup>-ATPase. *Annu Rev Physiol* 1996;58:427-445.
- 83 Purkerson JM, Schwartz GJ: The role of carbonic anhydrases in renal physiology. *Kidney Int* 2007;71:103-115.
- 84 Soleimani M, Grassl SM, Aronson PS: Stoichiometry of Na<sup>+</sup>/HCO<sub>3</sub><sup>-</sup> cotransport in basolateral membrane vesicles isolated from rabbit renal cortex. *J Clin Invest* 1987;79:1276-1280.
- 85 Skelton LA, Boron WF, Zhou Y: Acid-base transport by the renal proximal tubule. *J Nephrol* 2010;23:S4-S18.
- 86 Good DW: The thick ascending limb as a site of renal bicarbonate reabsorption. *Semin Nephrol* 1993;13:225-235.
- 87 Good DW, Watts BA3rd, George T, Meyer JW, Shull G: Transepithelial HCO<sub>3</sub><sup>-</sup> absorption is defective

- in renal thick ascending limbs from Na<sup>+</sup>/H<sup>+</sup> exchanger NHE1 null mutant mice. *Am J Physiol* 2004;287:F1244-F1249.
- 88 Alper SL, Stuart-Tilley AK, Biemesderfer D, Shmukler BE, Brown D: Immunolocalization of AE2 anion exchanger in rat kidney. *Am J Physiol Renal Physiol* 1997;273:F601-F614.
- 89 Good DW, George T, Watts BA3rd: Basolateral membrane Na<sup>+</sup>/H<sup>+</sup> exchange enhances HCO<sub>3</sub><sup>-</sup> absorption in rat medullary thick ascending limb evidence for functional coupling between basolateral and apical membrane Na<sup>+</sup>/H<sup>+</sup> exchangers. *Proc Natl Acad Sci USA* 1995;92:12525-12529.
- 90 Watts BA3rd, George T, Good DW: The basolateral NHE1 Na<sup>+</sup>/H<sup>+</sup> exchanger regulates transepithelial HCO<sub>3</sub><sup>-</sup> absorption through actin cytoskeleton remodeling in renal thick ascending limb. *J Biol Chem* 2005;280:11439-11447.
- 91 Watts BA3rd, George T, Good DW: Nerve growth factor inhibits HCO<sub>3</sub><sup>-</sup> absorption in renal thick ascending limb through inhibition of basolateral membrane Na<sup>+</sup>/H<sup>+</sup> exchange. *J Biol Chem* 1999;274:7841-7847.
- 92 Schelling JR, Nkemere N, Kopp JB, Cleveland RP: Fas-dependent fratricidal apoptosis is a mechanism of tubular epithelial cell deletion in chronic renal failure. *Lab Invest* 1998;78:813-824.
- 93 Khan S, Cleveland RP, Koch CJ, Schelling JR: Hypoxia induces renal tubular epithelial cell apoptosis in chronic renal disease. *Lab Invest* 1999;79:1089-1099.
- 94 Schainuck LI, Striker GE, Cutler RE, Benditt EP: Structural-functional correlations in renal disease. *Hum Pathol* 1970;1:631-641.
- 95 Wang Y, Wang YP, Tay YC, Harris DC: Progressive adriamycin nephropathy in mice: sequence of histologic and immunohistochemical events. *Kidney Int* 2000;58:1797-1804.
- 96 Bortner CD, Cidlowski JA: Absence of volume regulatory mechanisms contributes to the rapid activation of apoptosis in thymocytes. *Am J Physiol Cell Physiol* 1996;271:C950-C961.
- 97 Mc Manus ML, Churchwell KB, Strange K: Regulation of cell volume in health and disease. *N Engl J Med* 1995;333:1260-1266.
- 98 Okada Y, Maeno E, Shimizu T, Dezaki K, Wang J, Morishima S: Receptor-mediated control of regulatory volume decrease [RVD] and apoptotic volume decrease [AVD]. *J Physiol* 2001;532:3-16.
- 99 Schelling JR, Abu Jawdeh BG: Regulation of cell survival by Na<sup>+</sup>/H<sup>+</sup> exchanger-1. *Am J Physiol Renal Physiol* 2008;295:F625-F632.
- 100 Ginns SM, Knepper MA, Ecelbarger CA, Terris J, He X, Coleman RA, Wade JB: Immunolocalization of the secretory isoform of Na-K-Cl cotransporter in rat renal intercalated cells. *J Am Soc Nephrol* 1996;7:2533-2542.
- 101 Kaplan MR, Plotkin MD, Brown D, Hebert SC, Delpire E: Expression of the mouse Na-K-2Cl cotransporter, mBSC2, in the terminal inner medullary collecting duct, the glomerular and extraglomerular mesangium, and the glomerular afferent arteriole. *J Clin Invest* 1996;98:723-730.
- 102 Matsuyama S, Llopis J, Deveraux QL, Tsien RY, Reed JC: Changes in intramitochondrial and cytosolic pH: early events that modulate caspase activation during apoptosis. *Nat Cell Biol* 2000;2:318-325.
- 103 Tafani M, Cohn JA, Karpnich NO, Rothman RJ, Russo MA, Farber JL: Regulation of intracellular pH mediates Bax activation in HeLa cells treated with staurosporine or tumor necrosis factor. *J Biol Chem* 2002;277:49569-49576.
- 104 Wu KL, Khan S, Lakhe-Reddy S, Wang L, Jarad G, Miller RT, Konieczkowski M, Brown AM, Sedor JR, Schelling JR: Renal tubular epithelial cell apoptosis is associated with caspase cleavage of the NHE1 Na<sup>+</sup>/H<sup>+</sup> exchanger. *Am J Physiol Renal Physiol* 2003;284:F829-F839.
- 105 Cox GA, Lutz CM, Yang CL, Biemesderfer D, Bronson RT, Fu A, Aronson PS, Noebels JL, Frankel WN: Sodium/hydrogen exchanger gene defect in slow-wave epilepsy mutant mice. *Cell* 1997;91:139-148.
- 106 Khan S, Wu KL, Sedor JR, Abu Jawdeh BG, Schelling JR: The NHE1 Na<sup>+</sup>/H<sup>+</sup> exchanger regulates cell survival by activating and targeting ezrin to specific plasma membrane domains. *Cell Mol Biol [Noisy-le-grand]* 2006;52:115-121.
- 107 Manucha W, Carrizo L, Ruete C, Valles PG: Apoptosis induction is associated with decreased NHE1 expression in neonatal unilateral ureteric obstruction. *BJU Int* 2007;100:191-198.
- 108 Bocanegra V, Gil Lorenzo AF, Cacciamani V, Benardon ME, Costantino VV, Vallés PG: RhoA and MAPKinase signal transduction pathways regulate NHE1 Na<sup>+</sup>/H<sup>+</sup> exchanger-dependent proximal tubule cell apoptosis following mechanical stretch. *Am J Physiol Renal Physiol* 2014;307:F881-F889.
- 109 Wu KL, Khan S, Lakhe-Reddy S, Jarad G, Mukherjee A, Obejero-Paz CA, Konieczkowski M, Sedor JR, Schelling JR: The NHE1 Na<sup>+</sup>/H<sup>+</sup> exchanger recruits ezrin/radixin/moesin proteins to regulate Akt-dependent cell survival. *J Biol Chem* 2004;279:26280-26286.