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REVIEW

Na^+–H^+ exchanger-1 (NHE1) regulation in kidney proximal tubule

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Abstract The ubiquitously expressed plasma membrane Na^+–H^+ exchanger NHE1 is a 12 transmembrane-spanning protein that directs important cell functions such as homeostatic intracellular volume and pH control. The 315 amino acid cytosolic tail of NHE1 binds plasma membrane phospholipids and multiple proteins that regulate additional, ion-translocation independent functions. This review focuses on NHE1 structure/function relationships, as well as the role of NHE1 in kidney proximal tubule functions, including pH regulation, vectorial Na^+ transport, cell volume control and cell survival. The implications of these functions are particularly critical in the setting of progressive, albuminuric kidney diseases, where the accumulation of reabsorbed fatty acids leads to disruption of NHE1-membrane phospholipid interactions and tubular atrophy, which is a poor prognostic factor for progression to end stage renal disease. This review amplifies the vital role of the proximal tubule NHE1 Na^+–H^+ exchanger as a kidney cell survival factor.

Keywords Na^+–H^+ exchange · Ion transport · Apoptosis · Cell survival · Tubular atrophy · Chronic kidney disease

Abbreviations

AE2	Anion exchange protein 2
AKI	Acute kidney injury
CHP1	Calcineurin-homologous protein 1
CKD	Chronic kidney disease
ERM	Ezrin/radixin/moesin
ESRD	End stage renal disease
JAK2	Janus kinase 2
LC-CoA	Long chain fatty acyl-CoA
MDCK	Madin-Darby canine kidney cell line
NaPi-IIa	Na^+-phosphate co-transporter
NBC	Na^+/HCO_3^- co-transporter
NCX1	Na^+–Ca^{2+} exchanger 1
NHE	Na^+–H^+ exchanger
NIK	Nck (non-catalytic region of tyrosine kinase adaptor protein 1)-interacting kinase
NKCC1	$Na^+/K^+/Cl^-$ co-transporter 1
pH_i	Intracellular pH
PI(4,5)P2	Phosphatidylinositol 4,5-bisphosphate
ROCK1	Rho-associated, coiled-coil-containing protein kinase 1
RVI	Regulatory volume increase
SGK1	Serum and glucocorticoid-regulated kinase 1
SGLT	Sodium-glucose co-transporter
TM	Transmembrane-spanning domain

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Introduction

Discovery of Na^+–H^+ exchange

In 1966 Mitchell hypothesized the existence of an electroneutral transport mechanism that couples the exchange, or antiport, of cations and protons across the mitochondrial inner membrane [1]. Subsequent studies of bacterial membrane transport processes provided the first evidence of an electroneutral Na^+–H^+ exchanger [2, 3], and Na^+–H^+ exchanger activity was described shortly thereafter in mammals; specifically in apical membrane preparations from the small intestines and renal proximal tubules of rats [4].

The SLC9A family

The first mammalian Na^+–H^+ exchanger gene to be cloned, now known as *SLC9A1*, encodes the ubiquitously expressed, amiloride-sensitive Na^+–H^+ exchanger NHE1 (sometimes notated as NHE-1) [5]. *SLC9A1* maps to human chromosome 1p36.11 and to chromosome 4D2.3 in mice. We now know that the *SLC9A* gene family includes nine members encoding NHE1-NHE9,[1] all of which are *bona fide* Na^+–H^+ exchangers. NHE1-5 localize primarily to the plasma membrane, in contrast to NHE6-9 that reside in organelle membrane compartments [6, 7]. Of the plasma membrane NHEs, NHE1 and NHE2 are expressed in multiple tissues, whereas NHE3 is restricted primarily to kidney and intestine [8], NHE4 mainly to stomach and kidney [9] and NHE5 predominantly to brain, testis and spleen [10, 11]. Following the cloning of numerous Na^+–H^+ exchanger genes from multiple species, it is now appreciated that mammalian NHE proteins share no substantial amino acid sequence identity with their bacterial, fungal, or plantal counterparts. However, three-dimensional modeling predictions based on the crystal structure of a bacterial electrogenic Na^+–$2H^+$ antiporter (NhaA) [12] indicate that all NHEs are likely to adopt a similar three-dimensional conformation and thus may share common ancestry and transport mechanisms [13, 14].

The SLC9B and SLC9C families

The wider mammalian *SLC9* superfamily includes two other, smaller gene families: *SLC9B* and *SLC9C*. Each family includes two members, neither of which exhibit substantial sequence homology to NHEs of the *SLC9A* family. However, the two *SLC9B* family members do exhibit sequence similarity to cation/proton exchangers from lower organisms [15]. No functional data is available for the testes-expressed *SLC9B1* product, aka the Na^+–H^+ exchanger domain-containing protein NHEDC1 [16]. The *SLC9B2* product NHEDC2, also known as NHA2, exhibits a broader expression pattern and appears capable of NHE activity inasmuch as it promotes Na^+ tolerance at acidic extracellular pH when heterologously expressed in yeast [17]. In the kidney, NHA2 localizes to the distal convoluted tubule, where it has been speculated to play a role in blood pressure control [6, 17]. The *SLC9C1* product 'NHE10' is expressed in osteoclasts and sperm [18]. Although innate NHE10-regulated Na^+–H^+ activity has been difficult to demonstrate, when expressed as a chimeric protein that includes the first transmembrane span of NHE1, trafficking to the plasma membrane was enhanced and Na^+–H^+ exchange was detectable [19]. The function of *SLC9C2* is yet to be determined.

NHE1 action

Substrates and inhibitors

NHE1, in common with NHE2-5, mediates the electroneutral (1:1 stoichiometry) exchange of Na^+ and H^+ across the plasma membrane of cells, typically exploiting the inwardly directed Na^+ gradient established by the Na^+–K^+ ATPase to extrude H^+, especially when intracellular pH is acidic. NHE1 is quiescent in resting cells [20, 21], but can be activated by a variety of stimuli, as discussed later. The K_m for extracellular Na^+ is ~6–10 mM, and the pK_i (reporting the K_m for intracellular H^+ in pH units) is ~6.6–6.8 [22, 23]. [2] It has also been demonstrated that NHE1 is capable of operating in reverse mode [24], and also as a Na^+–Li^+ exchanger [25, 26]. NHE1, like NHE2-5, is inhibited by amiloride and its derivatives [e.g., 5-(N-ethyl-N-isopropyl)amiloride EIPA] [27], enabling the pharmacological distinction of NHEs from amiloride-insensitive Na^+/HCO_3^- co-transporters (NBCs), one of which (NBCe1-A) sits alongside NHE1 and regulates intracellular Na^+ and pH in the kidney proximal tubule [28]. Furthermore, NHE1 can be pharmacologically distinguished from other NHEs by virtue of differences in inhibitory constants for amiloride. For example, EIPA is at least one order of magnitude more selective for NHE1 (K_i ~ 0.02 μM) [29, 30] compared to any of the other plasma membrane expressed NHEs (K_i ~ 0.5–500 μM) [23, 29–33]; benzoyl guanidine derivatives, such as

[1] NHE3 is the transporter responsible for the activity originally detected in the apical membranes preparations from rat proximal tubules.

[2] The K_m for intracellular H^+ in pH units is sometimes referred to as pK_i. Note that 'pK_i' is not defined as the protonation state of titratable groups in the protein.

cariporide (aka HOE692) exhibit even greater NHE1 specificity (reviewed in Ref. [27]). The precise mode of action of these drugs is unknown, but mutagenesis studies reveal that hydrophobic residues in the vicinity of the fourth transmembrane-spanning domain (TM4, NHE1 residues 160–180)[3] of NHE1 are major, albeit not exclusive, determinants of amiloride and cariporide sensitivity [34–39]. It is interesting to note that a non-canonical splice variant of NHE1 from reticulocytes, which lacks this TM region, is thought to contribute to the amiloride-insensitive Na^+–Li^+ exchange activity evident in erythrocytes [26].

Mode of action

NHE1 is hypothesized to operate via an alternating access model whereby Na^+ binding to an extracellular NHE1 site triggers a conformational change that translocates Na^+ across the membrane. The NHE1 structural alteration simultaneously exposes a cytoplasmic substrate-binding site for intracellular H^+, permitting H^+ binding, reversal of the conformational change, and export of H^+ [40]. NHE1 forms homodimers with inter-monomer interfaces between adjacent TM domains and between adjacent cytosolic domains [41–43]. Component monomers within a dimer are capable of acting independently; inactivating mutations in one monomer do not exert a dominant negative effect on NHE1 activity, at least at acidic cytosolic pH [41, 44]. However, dimers may co-operate to perform coupled exchange (i.e., $2Na^+$–$2H^+$ exchange) at neutral or alkaline pH [44, 45], a mode of action that is supported by the crystal structures of the distantly related bacterial Na^+–H^+ antiporter NhaA [46]. NHE1 can also functionally couple with other transporters, influencing their actions and producing novel net transport functions. Examples of transporters influenced or functionally linked to NHE1 including Na^+–Ca^{2+} exchangers (NHE1 loads intracellular Na^+, thereby promoting Ca^{2+} influx) [47, 48], Cl^-–HCO_3^- exchangers (extruded H^+ and HCO_3^- titrate each other, resulting in net NaCl influx) [49], and the H^+-coupled peptide transporter PEPT2 (NHE1 disposes of intracellular H^+, promoting peptide influx) [50].

NHE1 distribution

The number of cell types that express NHE1 is so diverse that the transporter is often described as being ubiquitous. Reports of mammalian cell types that lack NHE1 (e.g., some dopaminergic neurons and microglia) [51, 52] are an exception, making immortalized cell lines that do not express NHE1 a valuable commodity for heterologous NHE1 expression studies [53]. Within the kidney, NHE1 exhibits the broadest distribution of the plasma membrane expressed NHEs, with documented expression in all nephron segments with the exception of the macula densa and intercalated cells of the distal nephron [54, 55]. In some cell types, NHE1 expression coincides with that of other NHEs. For example, proximal tubules express basolateral NHE1 and apical NHE2 and NHE3, while renal thick ascending limb epithelia express basolateral NHE1 and NHE4, as well as apical NHE2 and NHE3 [54, 56–58]. The half-life of plasma membrane NHE1 is relatively long ($\sim$24 h) and, unlike NHE3, NHE1 surface expression is not significantly regulated by trafficking or recycling [59–61]. However, a recent report demonstrated NHE1 expression may be regulated to some extent by ubiquitination and proteasomal degradation [62]. Although typically located in the basolateral membrane of diverse epithelia, NHE1 is found in the apical membrane of choroid plexus epithelia [63]. Besides diverse epithelia, other notable NHE1-expressing cell types include neurons, astrocytes [64], peripheral blood cells [65], myocytes [66], and sperm [67].

NHE1 structure–function

Domain structure

No high-resolution crystal structures exist for any SLC9 family members, but circular dichroism, electron paramagnetic resonance, nuclear magnetic resonance and mutagenesis studies have yielded some insights [68]. The biggest potential breakthrough has arisen from solving the structures of the bacterial Na^+–H^+ exchangers NhaA [12] and NapA [46]. Although sequence homology between bacterial Na^+–H^+ exchangers and mammalian NHEs at the amino acid level is very low, there are significant structural similarities, and efforts are underway to create a model NHE1 structure extrapolated from NhaA, which can then be reconciled with the wealth of NHE1 structure–function studies [13, 40, 69]. Extrapolation from NhaA confirms common features such as a short N-terminal cytosolic tail, an ion-translocating domain with 12 TMs, and a relatively long carboxy-terminal cytosolic domain that serves a regulatory function. The features of the NHE1 transport and regulatory domains are considered below and shown in Fig. 1.

N-terminal tail

This short 15 amino acid sequence, which extends into the cytosol, has no known role other than presumably to anchor TM1 in the membrane.

[3] Another determinant, Gly346 at the extracellular end of TM9, is hypothesized to be in three-dimensional proximity to the clustered determinants in TM4 [24].

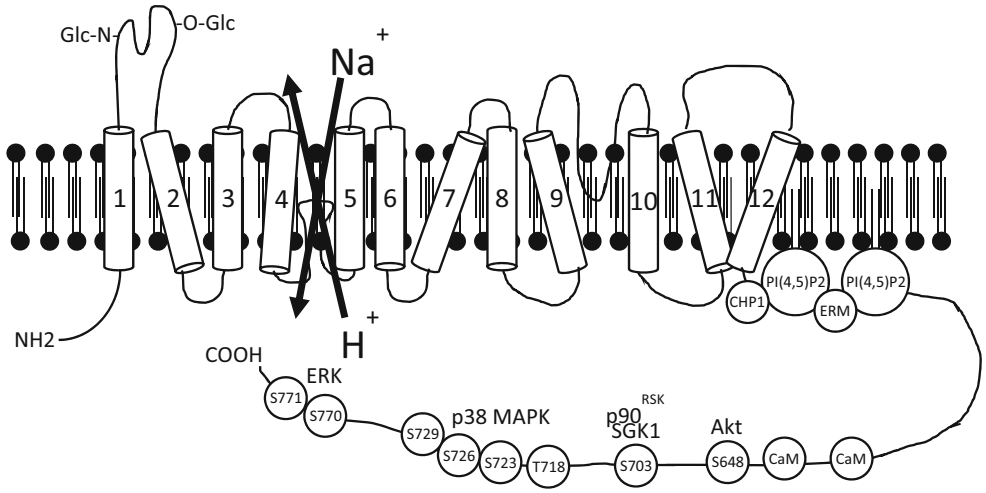

Fig. 1 Schematic diagram of NHE1 Na^+–H^+ exchanger structure. *Numbers* represent predicted transmembrane domains. Phosphorylation sites are depicted by the amino acid abbreviation and residue numbers. *CaM* calmodulin, *CHP1* calcineurin-homologous protein 1, *ERK* extracellular signal-related kinase, *ERM* ezrin/radixin/moesin, *MAPK* mitogen-activated protein kinase, $p90^{RSK}$ p90 ribosomal S6 kinase, *PI(4,5)P2* phosphatidylinositol 4,5-bisphosphate, *SGK1* serum and glucocorticoid-regulated kinase 1

Transmembrane-spanning ion-translocation domain

This 485 amino acid sequence is composed of 12 TMs joined by short loops as well as a long re-entrant loop that dips into the plane of the membrane between TM9 and TM10. The first extracellular loop that joins TM1 to TM2 contains both *N*- and *O*-linked glycosylation sites [70, 71], with *N*-glycosylation assisting in the targeting of mature NHE1 to the basolateral membrane of polarized epithelial cells [59]. Once at the plasma membrane, the extracellular loops joining TM1 to TM2 and TM3 to TM4 can be proteolytically cleaved without any obvious detriment to Na^+–H^+ exchange activity [71]. However, it has not been determined whether it is the entire region encompassed by TM1-TM3, or just the integrity of the extracellular loops in that region, that is dispensable for basal NHE1 activity.

Interpretations of current homology models indicate that TM4, in addition to being a major determinant of inhibitor sensitivity (see above), contributes residues that line the intracellular and extracellular substrate access pathways [13, 40]. Both models also predict that disordered regions in the middle of TM11 and one other span (either TM4 or TM6, depending on the model) come together to form the transport 'gate'/catalytic core that occludes the extracellular and intracellular substrate access pathways [13, 40]. However, the identity of the other spans that contributes to the access pathways of specific residues that form the substrate-binding sites remain controversial and are not readily reconciled by structure–function studies [69]. In addition to a substrate–H^+ binding site, residues in the vicinity of the cytoplasmic loops that join TM2 to TM3 and TM10 to TM11 contribute to allosteric regulation of NHE1 by pH_i (activation by H^+), either by directly supporting the action of an allosteric H^+-modifier site or by influencing the interaction of the membrane domain with the carboxy-terminal regulatory domain, which itself can influence the set-point of the modifier site [22, 72].

Carboxy-terminal regulatory domain

The 315 amino acid cytosolic carboxy-terminal domain of NHE1 is the site of numerous regulatory events that can activate NHE1. The initial juxtamembrane portion of the carboxy terminus contains two polybasic motifs (at residues 513–520 and 556–564 of NHE1) that bind inner leaflet phosphoinositides such as phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2] [73–77], consistent with NHE1 compartmentalization to lipid rafts [78–80], which may be enriched for phosphoinositides [81]. The cytoskeletal adaptor proteins ezrin/radixin/moesin (ERM) bind the same NHE1 residues [82–84], but this interaction, as well as downstream ramifications, appear to be independent of Na^+–H^+ translocation, whereas PI(4,5)P2 binding to the same sites is required for Na^+–H^+ exchange [73, 82, 83]. The 4.1 adaptor protein family shares sequence homology to ERM, and multiple splice variants are expressed in kidney, including 4.1B in the proximal tubule and 4.1R in the thick ascending limb of Henle's loop [85]. The 4.1R isoform interacts with the same C-terminal NHE1 residues as ERM and PI(4,5)P2, with $K_D = 100$–200 nM [86]. Calmodulin binding to NHE1 (see below) lowers the affinity of the 4.1R-NHE1 interaction, which permits PI(4,5)P2 binding and NHE1 activation. Experiments using

NHE1 constructs with K/A and R/A mutations at the 513–520 and 556–564 sites, as well as ATP depletion to modulate PI(4,5)P2 levels, indicate that PI(4,5)P2 binding elicits an alkaline shift in NHE1 K_m for intracellular H^+, thereby causing an alkaline shift in pH_i [73]. Although the activating NHE1–PI(4,5)P2 interaction has been confirmed by many labs, the selectivity of NHE1 binding to other phospholipids remains unclear. One report, using co-sedimentation methods, demonstrated promiscuous binding between an NHE1 cytosolic domain polypeptide and PI(4,5)P2, phosphatidylinositol, phosphatidylserine, phosphatidylglycerol and phosphatidic acid [74]. Another report, using membrane overlay assays and surface plasmon resonance, identified specific interactions between th NHE1 cytosolic domain and phosphatidylinositol polyphosphates, with negligible binding between NHE1 and other membrane phospholipids [75]. In addition to differences in assay methods, the NHE1 polypeptide sequences used for the binding studies differed—mouse peptide, residues 546–602 [74]; rat peptide, residues 501–815 [75]— which may account for some discrepancy. The NHE1-phosphoinositide interactions are low affinity [5.2×10^{-5} M for PI(4,5)P2; 2.5×10^{-5} M for PI(3,4,5)P3] [75], which is likely to be relevant in vivo, by facilitating rapid on–off binding, as opposed to higher affinity interactions, which tend to be irreversible.

The Ca^{2+}-stimulated binding of either calcineurin-homologous protein CHP1 (to residues 515–530 of NHE1; [87–89]) or calmodulin (to residues 636–656; [90–93]) also enhances NHE1 activity, by eliciting an alkaline shift in K_m for intracellular H^+. In the case of calmodulin, this occurs by the masking of an autoinhibitory domain in the cytosolic tail that, when unoccupied, promotes an acidic shift in the pH-dependent K_m for NHE1 [91, 92]. A truncated NHE1 that lacks the distal cytosolic tail also exhibits a substantially more acidic K_m for intracellular H^+ than the intact protein [94, 95], perhaps due to loss of the influence of several phosphorylatable serine and threonine residues. These sites are constitutively phosphorylated in quiescent cells [96], but can then be further phosphorylated in response to many extracellular stimuli, including growth factors, hormones, extracellular matrix–integrin interactions, and sustained intracellular acidosis (Fig. 1) [97]. NHE1 phosphorylation by MAP kinases [98–100], $p90^{RSK}$ [101], NIK [102], ROCK1 [103], JAK2 [93], Akt [104, 105] and SGK1 [106] regulate many cell phenotypes, such as proliferation [107–109], differentiation [110, 111], adhesion [112] and migration [113, 114]. An exception is NHE1 activation by extracellular hypertonic stimuli, which does not require NHE1 phosphorylation [115, 116]. Many of the phosphorylation and binding partner studies have previously been extensively reviewed [47, 117, 118].

Cellular mechanisms of NHE1 function

pH regulation

NHE1 harnesses the inwardly directed Na^+ gradient to remove H^+ from the intracellular milieu, thereby resisting acidosis. Virtually all physiological processes are pH sensitive and the importance of NHE1 in the protection of these processes is reflected in its near ubiquitous expression and frequent reference to the role of NHE1 as an important housekeeping protein. A critical NHE1 function, which is discussed below, is resistance to proximal tubule apoptotic stress in the context of chronic kidney diseases (CKDs), such as diabetic nephropathy. A universal feature of apoptosis is the activation of endonucleases and executioner caspases, which catalyze degradation of DNA and intracellular proteins, respectively. Many of these enzymes are maximally catalyzed at pH values that are encountered in apoptosis, but well below physiologic intracellular pH range, which serves as a safeguard to prevent accidental activation, with lethal consequences [119–122]. By perpetually extruding H^+, in exchange for Na^+, NHE1 plays an important housekeeping role by maintaining homeostatic intracellular pH that is sufficiently high to allow optimal function of most enzymes and structural proteins, while preventing activation of endonucleases and caspases.

Additional examples of the impact of NHE1 action on pH are maintenance of neuronal excitability by influence upon pH-sensitive ion channels and receptors (reviewed in [123]), support of the inflammatory response by disposing of the intracellular acid load that accompanies the respiratory burst in immune cells (reviewed in [124]), and promotion of cell migration by influencing pH-dependent protein–protein interactions within focal adhesion complexes that link the cytoskeleton to the extracellular matrix. In the case of migration, NHE1 is recruited to focal adhesion complexes, and an interaction with integrins stimulates NHE1 action, thereby creating pH nanodomains around these structures [125, 126]. The NHE1 action generates an intracellular alkaline nanodomain that could influence actin remodeling and promote focal adhesion turnover at the leading edge of migrating cells [127, 128], but also causes a complementary extracellular acidic nanodomain that strengthens the interaction between integrins and their extracellular matrix ligands [129]. NHE1 activity also facilitates migration by promoting the degradation of matrix proteins via enhancement of the expression and activity of pericellular matrix metalloproteinases [130, 131].

Vectorial Na^+ transport

A major function of the kidney is maintenance of extracellular fluid volume, and this is mediated predominantly

through proximal tubule isosmotic reabsorption of 50–90 % of filtered Na^+. Because of the large amounts of solute and water transported by the proximal tubule, considerable coordination is required between multiple apical and basolateral transporters to maintain cell volume and pH (see Fig. 2).

Of the luminal Na^+ transporters NHE3 is responsible for the greatest quantitative uptake of Na^+ from ultrafiltrate, with most reabsorption occurring within the initial S1 segment [132, 133]. NHE2 is also expressed in the proximal tubule brush border, but in comparative studies with microperfused proximal tubules derived from NHE2 and NHE3 knockout mice, relatively little Na^+–H^+ translocation was mediated by NHE2 [134]. The Na^+–glucose transporters, SGLT1 and SGLT2 are expressed within proximal tubule brush border, and also contribute to luminal Na^+ reabsorption. SLGT1 is a high affinity, low capacity transporter, with a 2:1 stoichiometry for Na^+ and glucose. SGLT2 is low affinity, high capacity, and transports Na^+ and glucose in a 1:1 ratio. The NaPi-IIa Na^+-phosphate co-transporter is a minor contributor to luminal Na^+ uptake, but a major effector of inorganic phosphate reabsorption, with approximately 80 % of filtered phosphate reabsorbed by proximal tubule NaPi-IIa, which is localized primarily in the S1 segment brush border [135].

The low cytosolic Na^+ and high K^+ relative to plasma concentrations is largely attributable to Na^+–K^+ ATPase activity, which is localized exclusively to the basolateral proximal tubule membrane. As shown in Fig. 2, the Na^+ pump is the major basolateral Na^+ transporter, and is highly regulated by intracellular Na^+ concentration [136], implying that luminal Na^+ uptake represents the rate-limiting step in net proximal tubule Na^+ reabsorption. The Na^+/HCO_3^- co-transporter NBCe1-A, which localizes to the basolateral membrane, is also a source of proximal tubule Na^+ reabsorption, and the major pathway for HCO_3^- exit across the peritubular membrane [137]. The relatively small contribution of NHE1 to basolateral Na^+ transport highlights the importance of its biological functions that are mediated by ion transport-independent mechanisms. However, in contrast to proximal tubule NHE3 and Na^+–K^+ ATPase, which are responsible for large transcellular Na^+ fluxes to maintain extracellular fluid volume, relatively small changes in NHE1-regulated Na^+ flux can have a large impact on other physiologic functions, such as maintenance of cell volume (discussed later).

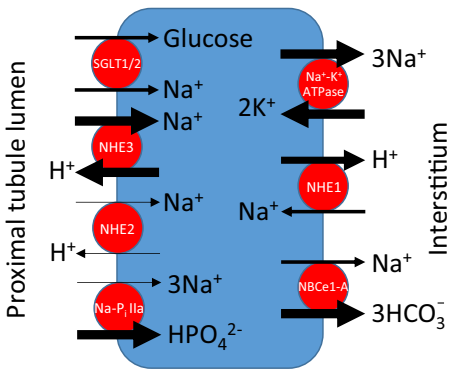

Fig. 2 Major proximal tubule Na^+ transporters. For simplicity, "pure" anion transporters, paracellular pathways, and distinction between S1, S2 and S3 proximal tubule segments have been omitted. The thickness of the *arrows* is intended to reflect the relative, quantitative transport of the indicated ion. *NHE* Na^+–H^+ exchanger, *NaP_i IIa* renal Na^+-phosphate co-transporter, *NBCe1-A* electrogenic Na^+/HCO_3^- co-transporter

Although the direction of NHE1-dependent Na^+ flux is opposite to Na^+–K^+ ATPase and NBCe1-A mediated Na^+ translocation, NHE1 may play a regulatory role in proximal tubule vectorial Na^+ transport through functional interaction with these transporters. Stimulation of proximal tubule Na^+–K^+ ATPase concomitantly increased NHE1 expression and activity, as well as physical association of NHE1 with the Na^+–K^+ ATPase α1 subunit [138]. NHE1 was required for Na^+–K^+ ATPase activity, as well as α1 subunit phosphorylation and Na^+ pump trafficking to the plasma membrane. The mechanism by which NHE1 enhances proximal tubule Na^+–K^+ ATPase was not identified, but the authors noted that it was unlikely to be related to Na^+ entry across the basolateral membrane, and speculated that dual phosphorylation by common kinases could foster protein–protein interactions, or that non-transport NHE1 scaffolding properties could be responsible [138].

Despite co-localization to the basolateral membrane, and common teleology for regulating intracellular Na^+ and pH, we are unaware of functional interactions between NHE1 and NBCe1-A in the proximal tubule. However, cooperation between NHE1 and NBCe1 has been described in cardiac tissue. The two transporters are spatially separate in the heart (NHE1 is expressed at the intercalated disc and gap junctions; NBCe1 and NBCn1 are expressed in transverse tubules), but they nevertheless coordinately facilitate Na^+ influx, intracellular alkalinization, and ultimately Ca^{2+} loading of the sarcoplasmic reticulum for excitation–contraction coupling [139]. In studies of NHE1-deficient MDCK cells, which more closely resemble distal, rather than proximal tubule cells, NBCe1 expression was upregulated, which compensated for defects in intracellular volume and pH regulation [140]. However, a cell migration phenotype was not rescued, indicating that not all NHE1 and NBCe1 functions are redundant.

Cell volume regulation

NHE1 contributes to a cell volume control mechanism—regulatory volume increase (RVI)—under hypertonic stress

NHE1 promotes the net uptake of osmolytes, and thence water, thereby tending to counter cell shrinkage (reviewed in [141]). Other transporters have been implicated in RVI, such as NKCC1 $Na^+/K^+/2Cl^-$ and the AE2 Cl^-–HCO_3^- exchangers [142, 143], but neither is expressed in the proximal tubule [144, 145], thereby rendering NHE1 as a major regulator of proximal tubule RVI. Interestingly, in proximal tubular epithelial cells, as well as fibroblasts, acute cell shrinkage causes opposite effects on NHE1 (activation) and NHE3 (inhibition) [78, 146, 147]. In the case of NHE1, extracellular hypertonicity increases tyrosine kinase activity in challenged cells that signal via a number of intermediates, such as JAK2 and calmodulin, to activate NHE1 via the C-terminal portion of its regulatory cytosolic domain [95, 148]. The extrusion of H^+ results in an increase in intracellular HCO_3^- concentration that then stimulates Cl^-–HCO_3^- exchange in AE2-expressing cell types. Because changes in intracellular pH and HCO_3^- are buffered, the counter-transported Na^+ and Cl^- produce a net increase in intracellular osmolality that promotes RVI.

The in vivo relevance of RVI to the proximal tubule has been difficult to discern, though it is plausible that constant fine tuning of cell volume is important for multiple intracellular processes, such as regulation of cell membrane curvature and proper approximation between interacting intracellular proteins. As discussed in the next section, RVI may also be critical for counteracting multiple cell volume perturbations, including apoptotic shrinkage [143, 149]. NHE1 has been implicated in pathophysiology of cell hypertrophy, particularly involving the cardiomyocyte, in the context of congestive heart failure [118]. In circulating leukocytes, studies that originated primarily from Sergio Grinstein's lab indicated a role for NHE1, both in cell volume and intracellular pH changes associated with phagocytosis [148, 150, 151]. Moreover, phagocytic cup formation is mediated by dynamic changes in plasma membrane morphology and cell volume, which require electrostatic interactions between anionic membrane phospholipids and poly-cationic intracellular proteins [152]. Although NHE1 has not specifically been shown to be a phospholipid binding partner for phagocytosis, we speculate that it is a plausible candidate, considering its known interaction with inner leaflet membrane phospholipids [74, 75].

NHE1 function at the systemic level

Genetic studies

In 1997 Cox et al. [153] characterized mice with an ataxia phenotype that was caused by a spontaneous NHE1 mutation of residue 441, between TM11 and TM12, which resulted in the insertion of a premature stop codon and unstable mRNA encoding a transporter without the C-terminal tail. These mice die within a few weeks of birth due to lethal seizures. Electroencephalography revealed a "slow wave epilepsy" pattern, hence the moniker *Swe/Swe* for these mice. Shortly thereafter Bell et al. [154] reported a virtually identical phenotype in mice with global NHE1 gene deletion. In contrast to NHE3 knockout mice or humans with loss of function NBCe1 mutations, which result in proximal tubule salt and HCO_3^- wasting [155, 156], neither *Swe/Swe* nor NHE1 knockout mice demonstrate a renal phenotype.

Human NHE1 mutations had not been identified until recently, when a non-synonymous Gly305Arg substitution was shown to cause Lichtenstein-Knorr syndrome, a rare, autosomal recessive disorder characterized by ataxia and neurosensory deafness, with onset of symptoms typically by 1–2 years of age [157]. The mutation removes a critical NHE1 glycosylation site, resulting in loss of NHE1 targeting to the plasma membrane and a consequent absence of NHE1 Na^+–H^+ exchange activity.

A recent report describes that gene deletions of either NHE1 or huntingtin, the gene mutated in Huntington's disease, resulted in a similar chemotaxis phenotype in *Dictyostelium* amoebae in response to extracellular K^+-regulated cAMP and Ca^{2+} stimuli [158]. Although this is consistent with the well-established role of NHE1 in cell migration [113], the novel finding is that in huntingtin-null organisms, actin filaments were disorganized, resulting in defective NHE1 trafficking to the plasma membrane [158]. Instead of normal expression at the leading edge of migrating cells, NHE1 was mislocalized to a perinuclear region in huntingtin knockout cells. Taken together, these data imply that there is a functional relationship between huntingtin and NHE1. However, human NHE1 mutations in Huntington's disease have not been described.

The reason for predominant brain phenotypes in NHE1-deficient, despite ubiquitous expression, is unclear. We speculate that the threshold may be lower for neuron dysfunction when some of the previously mentioned NHE1 functions, such as homeostatic intracellular pH or intracellular volume control are aberrant, perhaps due to less redundancy with NHE1-regulated pathways.

Kidney proximal tubule NHE1 function in animal models

Although neither the NHE1-deficient mice nor humans with loss of function NHE1 mutations display an overt renal phenotype, multiple groups have utilized animal models to test the effects of a stressor "second hit" to unmask NHE1 functions in vivo. In studies designed to identify the role of NHE1 in tubular atrophy, a critical

pathologic predictor of chronic, progressive kidney diseases [159–161], which is regulated by apoptosis [162], Wu et al. [149] showed that *Swe/Swe* mice receiving tail vein injections of adriamycin, a chemotherapeutic agent that is toxic to murine glomerular epithelial cells, developed an augmented renal phenotype that included proximal tubular epithelial cell apoptosis. *Swe/Swe* mice injected with streptozotocin to induce diabetes, developed hallmarks of diabetic nephropathy, including albuminuria, azotemia, and tubular epithelial cell apoptosis [84]. Importantly, the *Swe/Swe* mice were bred onto a C57BL/6 genetic background, which is resistant to adriamycin and streptozotocin toxicity, indicating that the "two hit" combination of NHE1 loss of function and glomerular injury was sufficient to overcome the protective C57BL/6 background. In a rat model of obstructive nephropathy, the resulting tubular epithelial cell apoptosis was associated with diminished NHE1 expression, and pharmacologic inhibition of NHE1 enhanced apoptosis [163]. Recent in vitro studies suggest that one mechanism for NHE1 suppression is through mechanical stretch-induced RhoA and MAP kinase activation [164]. Taken together, multiple studies in models of CKD suggest that tubular epithelial cell NHE1 is cytoprotective [47].

NHE1-regulated mechanisms of proximal tubule epithelial cell survival

Further support of NHE1 in the defense against apoptosis includes multiple in vitro studies demonstrating that apoptotic stress activates NHE1 [47, 83, 84, 149]. An invariant feature of apoptosis is cell volume decrease, and as previously mentioned, NHE1 might relieve apoptotic stress through activation of RVI pathways [143, 149]. Apoptotic cells also undergo cytosol acidification, which catalyzes pro-apoptotic enzymes [120, 121, 165, 166], suggesting that NHE1-regulated Na^+–H^+ exchange, which is regulated by the N-terminal, transmembrane domain of NHE1, might defend against renal tubular epithelial cell apoptosis by alkalinizing cytosolic pH, as well as expanding cell volume.

As mentioned previously, two polybasic juxtamembrane domains anchor NHE1 to the plasma membrane inner leaflet through binding to PI(4,5)P2 [73–75, 78] and ERM adaptor proteins [82, 83]. Both ERM and PI(4,5)P2 are substrates for PI-3 kinase, and the PI(3,4,5)P3 product docks the pro-survival kinase Akt, leading to its activation and phosphorylation of downstream targets that block apoptosis. This scaffolding function of NHE1, which is independent of Na^+–H^+ exchange activity, represents one mechanism of proximal tubule defense against apoptotic stress and tubular atrophy [75]. NHE1 activation of PI-3 kinase and Akt has also been implicated in glomerular epithelial cell (podocyte) survival, although in this case, the downstream effects of Akt activation are augmentation of autophagy and reduction in endoplasmic reticulum stress [167].

Consistent with the notion that NHE1 acts as a proximal tubule cell survival factor, multiple studies have demonstrated that tubular epithelial cell NHE1 becomes inactivated during apoptosis [83, 149, 163, 164]. However, the mechanisms of inactivation have been elusive until recently. Initial studies indicated that the NHE1 cytosolic tail undergoes caspase-3-dependent degradation [149, 168], but there are no consensus caspase cleavage sequences within the cytosolic tail, and relevant non-consensus sites have not been mapped. Decreased NHE1 mRNA and protein expression was noted in ureteral obstruction models [163, 164]. More recently, using animal models of kidney diseases characterized by glomerular damage and albuminuria, Khan et al. [76] have postulated that the aberrantly filtered albumin-bound fatty acids are reabsorbed by the proximal tubule. The intracellular fatty acids are then rapidly catalyzed to long chain acyl-CoA (LC-CoA), in preparation for β-oxidation and ATP generation (Fig. 3). However, the rate-limiting enzyme for LC-CoA transport into mitochondria, carnitine palmitoyl transferase-1a (CPT1a) becomes saturated by the large LC-CoA flux, resulting in extensive lipid droplet deposition within the proximal tubule cytoplasm [76, 169, 170]. Although the lipid droplets represent a non-toxic intracellular depot, the storage capacity in non-adipocytes is limited. The overflow LC-CoA, which bear structural similarity to PI(4,5)P2 bind the NHE1 cytosolic tail with greater affinity compared to PI(4,5)P2 [76]. If LC-CoA reach sufficiently high (low-mid μM) intracellular concentration, they compete with PI(4,5)P2 for binding to NHE1, and uncoupling of the NHE1-PI(4,5)P2 interaction leads to loss of NHE1 activity [76] (Fig. 3). In this scheme, proximal tubule NHE1 fulfills a unique role by serving as a metabolic sensor for lipotoxicity.

Acute kidney injury

The role of proximal tubule NHE1 appears to be quite different in the pathophysiology of acute kidney injury (AKI), which is most commonly induced by ischemia (reviewed in [47]). In particular, the S3 proximal tubule segment, which delicately balances high O_2 demand and low basal O_2 tension in the cortico-medullary region, is the nephron portion most vulnerable to ischemia. The divergent mechanisms of NHE1 in the pathophysiology of CKD and AKI are illustrated by reports that NHE1 inhibitors improve renal blood flow and ameliorate the clinical course of ischemic AKI [171, 172], whereas NHE1 inhibition exacerbates apoptosis in the context of CKD [75, 76, 83,

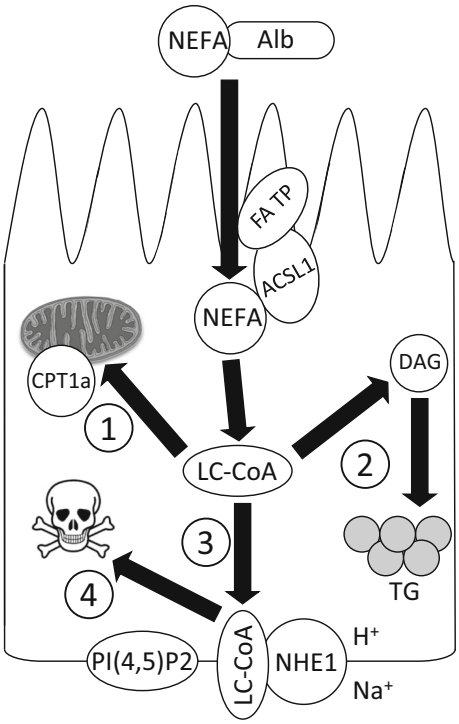

Fig. 3 Proposed sequence of events following aberrant fatty acid reabsorption at the apical surface of the proximal tubule in the setting of nephrotic syndrome. The *numbers* signify the order of metabolic pathway activation. *ACSL1* long chain acyl-CoA synthetase-1, *Alb* albumin, *CPT1a* carnitine palmitoyl transferase-1a, *DAG* diacylglycerol, *FA TP* fatty acid transport protein, *LC-CoA* long chain fatty acyl-CoA, *NEFA* non-esterified fatty acid, *NHE1* Na^+–H^+ exchanger-1, *PI(4,5)P2* phosphatidylinositol 4,5-bisphosphate

149, 163]. A plausible explanation for the discrepancy is that in CKD the stimulus for NHE1 activation is likely cell volume shrinkage due to an apoptotic stimulus, whereas in ischemic conditions anaerobic metabolism causes intracellular acidosis, which triggers NHE1 activity. While Na^+ influx in shrunken cells may restore cell volume and function (in CKD), Na^+ and H_2O movement into proximal tubule cells with normal volume leads to swelling, which is a cardinal feature of necrosis (in AKI). In ischemic cells that express the NCX1 Na^+–Ca^{2+} exchanger, NCX1 is activated in the reverse mode, to extrude excess intracellular Na^+, which can then perpetuate Ca^{2+}-dependent necrosis and apoptosis pathways (reviewed in [47]).

Conclusions

NHE1 is a ubiquitously expressed ion exchanger, which regulates electroneutral Na^+–H^+ translocation that is critical for many cell functions, most notably maintenance of intracellular pH and cell volume. NHE1 was the initially discovered Na^+–H^+ exchanger over 25 years ago, and is commonly and perhaps pejoratively referred to as a "housekeeping protein", implying that it is uninteresting or unworthy of scientific inquiry. However, extensive mapping and functional studies involving the regulatory NHE1 cytosolic domain have revealed that multiple protein and lipid binding partners direct an expanding list of NHE1 housekeeping chores. Among these is relief of proximal tubule apoptotic stress and CKD progression, which is accomplished by a novel mechanism, whereby NHE1 serves as a metabolic sensor for aberrantly accumulated fatty acid metabolites. We are optimistic that NHE1-regulated pathways may be exploited for further investigation of the pathophysiology of tubular atrophy, since it is a strong predictor of CKD progression, for which there are currently no specific diagnostic tests or therapies.

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