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Na⁺/H⁺ exchangers: physiology and link to hypertension and organ ischemia

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Abstract

Purpose of review—Na⁺/H⁺ exchangers (NHEs) are ubiquitous proteins with a very wide array of physiological functions, which are summarized here with an emphasis on the most recent advances. Hypertension and organ ischemia are two disease states of paramount importance in which NHEs have been implicated. The involvement of NHEs in the pathophysiology of these disorders is incompletely understood. This paper reviews the principal findings and current hypotheses linking NHE dysfunction to hypertension and ischemia.

Recent findings—With the advent of large-scale sequencing projects and powerful in-silico analyses, we have come to know what is most likely the entire mammalian NHE gene family. Recent advances have detailed the roles of NHE proteins, exploring new functions such as anchoring, scaffolding and pH regulation of intracellular compartments. Studies of NHEs in disease models, though not conclusive to date, have contributed new evidence on the interplay of ion transporters and the delicate ion balances that may become disrupted.

Summary—This review provides the interested reader with a concise overview of NHE physiology, and addresses the implication of NHEs in the pathophysiology of hypertension and organ ischemia in light of the most recent literature.

Keywords

ischemia; Na⁺/Ca²⁺ exchanger; Na⁺/H⁺ exchanger; NCX; NHE; primary hypertension; reperfusion

Introduction

Intrinsic to life is order. The strife for low entropy is a thermodynamically costly process and the maintenance of transmembrane ion gradients requires energy. Primary active transport involves direct coupling of ionic movement to the hydrolysis of high energy phosphate bonds. The transmembrane electrochemical potential difference thus created becomes the currency of energy for secondary active transport. The ability to exchange protons (H⁺) for sodium ions (Na⁺) across lipid bilayers by a secondary active mechanism is pervasive in all living organisms. Mammalian Na⁺/H⁺ exchangers (NHEs) catalyze the countertransport of Na⁺ and H⁺ across both plasma and organellar membranes.

Peter Mitchell first suggested the existence of cation/H⁺ exchange in the mitochondrial membrane in a pioneering monograph in 1961 [1] and provided experimental evidence for such an exchange process [2,3]. To date, genes encoding NHEs have been cloned from the simplest prokaryotes to the most advanced multicellular eukaryotes [4••]. The human genome encodes nine NHE isoforms (SLC9A1-9 genes, NHE1-9 proteins) that have different tissue and sub cellular distributions, in addition to a putative Na⁺/H⁺ exchanger with restricted expression in spermatozoa (SLC9A10 gene, sperm NHE protein). Given the ubiquity of this class of proteins (Table 1) and their extremely diverse functions (Fig. 1), it is not surprising that NHEs are implicated in multiple physiological and pathophysiological processes.

Mammalian Na⁺/H⁺ exchangers

The reader is referred to Table 1 for a summary of the tissue and cellular expression of NHEs. Their distribution in the kidney is detailed in Fig. 2.

NHE1 was the first mammalian NHE identified, in 1989 [11], and is probably the most important isoform for intracellular pH, cell volume and intracellular sodium homeostasis. NHE1 has been shown to regulate cell cycle, proliferation, migration and adhesion, and to confer resistance to apoptosis [12,13•]. NHE1 mediates transepithelial transport in secretory parotid acinar cells [14] and may mediate ammonium reabsorption in the thick ascending limb of the loop of Henle. Apart from its ionexchange function, NHE1 interacts with the ezrin, radixin and moesin (ERM) family of actin-binding proteins, serving as a membrane anchor for the actin cytoskeleton [15] and as a scaffold for the assembly of signaling complexes [16•]. NHE1 may also modulate other transporters, such as apical membrane NHE3, possibly through actin cytoskeleton remodeling [17,18•,19•]. Further insight into NHE1 function is offered by two NHE1-defective mouse models (Table 2).

NHE2 is important for Na⁺ secretion in the parotid gland, and plays a role in maintaining parietal cell integrity in the stomach (Table 2). In the kidney, NHE2 functions in transepithelial NaHCO₃ absorption in the distal nephron. At the macula densa NHE2 is the only luminal NHE, but its role in sodium sensing remains to be determined [34].

NHE3 differs from other isoforms in that it recycles between the plasma membrane (apical membrane of epithelia) and the endosomal compartment, and functions in both locations [35]. NHE3 activity is regulated by alterations in its intrinsic activity and by trafficking between these two compartments [36-42]. NHE3 is responsible for a significant portion of renal and intestinal Na⁺ absorption (Table 2). The absorptive and secretory functions of luminal membrane NHE3 in the kidney are summarized in Fig. 1b. Intracellular NHE3 has been postulated to be important for endosomal acidification in the reabsorption of filtered proteins by receptor-mediated endocytosis [43].

NHE4 is involved in intracellular pH and volume regulation, especially in some cells lacking NHE1 (Table 1). NHE4 may mediate ammonium transport from the thick ascending limb lumen to the interstitium, but this theory has not been explored with the NHE4^{-/-} mouse. Very little is known about the function of the neuron-specific isoform NHE5. The ubiquitously expressed NHE6, NHE7 and NHE9 likely serve as organellar pH regulators [9].

NHE8 was recently identified by in-silico cloning [44]. NHE8 may recycle to the plasma membrane and be regulated by trafficking, similar to NHE3, but there are no available data as yet. In the kidney, NHE8 may functionally complement NHE3 (Fig. 2). Although NHE3^{-/-} mice have no significant alteration in NHE8 protein expression [7•], the role of NHE8 in maintaining proximal sodium transport cannot be ruled out.

The sperm-specific NHE is a predicted hybrid protein expressed in spermatozoa, essential for sperm motility and male fertility [33]. Its Na^+/H^+ -exchange ability has not yet been demonstrated.

In spite of the impressive amount of knowledge that we have accumulated in the last years, including the completion of the Human Genome Project, the exact identity of the protein responsible for Peter Mitchell's mitochondrial cation/ H^+ exchange has remained elusive [45, 46].

Na^+/H^+ exchangers in hypertension

The role of NHEs in hypertension is a question of pivotal importance. There is a massive body of literature on this topic that is not easily amenable to any attempts at a summary. Several broad statements can be made regarding NHEs and hypertension. The majority of findings in this vast literature are correlations of the hypertensive state with either direct measurements of NHE (activity, protein, transcript) in animal models, or surrogates of NHE (Na^+/Li^+ or Na^+/H^+ exchange in erythrocytes, lymphocytes, platelets) in humans. There is little or no reassurance that the surrogates actually reflect NHE and, even if they do, causality and significance are difficult to establish. Observations have been made in animal models of polygenic hypertension that demonstrate changes in NHE preceding the hypertensive state. These types of observation rule out NHE defects being results of hypertension but still do not distinguish the difference between causation and parallel phenomena.

The current models of possible involvement of NHEs in hypertension are centered mainly on two isoforms, NHE1 and NHE3 (Fig. 3). Transgenic overexpression of NHE1 leads to salt-sensitive hypertension in mice [47]. Primary hypertension has been associated with increased NHE1 activity measured in both experimental animals and peripheral blood cells from some human subjects with primary hypertension [48]. The NHE1 locus has been ruled out as a candidate in essential hypertension by genetic linkage analysis [49]. When present, the defect is rather due to regulatory mechanisms that are, as yet, poorly defined.

One potential mechanism linking NHE1 to hypertension is overactivation of NHE1 in vascular smooth muscle cells (VSMCs), resulting in intracellular Na^+ accumulation, slowing down or reversal of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX), increased cytosolic Ca^{2+} concentration, and VSMC contraction. Chronically increased NHE1 activity may also drive abnormal VSMC growth and proliferation. There are several new arguments supporting the reversal of NCX exchange. The NCX inhibitor SEA0400 decreases systolic blood pressure in several rat models of salt-dependent hypertension [50•]. While $\text{NCX1}^{-/-}$ homozygosity is lethal, heterozygous $\text{NCX1}^{+/-}$ mice are viable and resistant to salt-dependent hypertension. Conversely, transgenic mice with VSMC-specific overexpression of NCX1 are more prone to high-salt-induced hypertension [50•]. However, there is no definitive evidence that NCX in VSMCs is slowed down or reversed as a result of NHE overactivity. There are alternative, non-mutually exclusive theories, such as the action of cardiotonic steroids (e.g. endogenous ouabain), which can cause intracellular Na^+ accumulation by inhibition of the Na^+/K^+ -ATPase.

Another major mechanism linking NHEs and hypertension could be renal alteration of sodium homeostasis and defective pressure natriuresis. $\text{NHE3}^{-/-}$ mice are hypotensive, even when small-intestine NHE3 is rescued by transgenic expression (Table 2). Conversely, overactivation of NHE3 could be a mechanism for hypertension. NHE3 has been studied in various rodent hypertension models (Table 3), and has been found to be implicated in some but not all the models. NHE3 regulatory proteins have also been studied in some models, with intriguing but inconclusive results [55]. Several polymorphic variants of NHE3 did not associate with human primary hypertension in a recent case-control study [61].

Although there is no definitive evidence documenting an NHE defect in human hypertension, both NHE1 and NHE3 remain plausible candidates and more studies are needed to clarify their involvement, as well as the possible involvement of other NHE isoforms.

Na⁺/H⁺ exchangers in ischemia/reperfusion injury

Ischemia/reperfusion injury is an extremely complex, incompletely understood phenomenon. A simplified working paradigm is that ischemia deprives energy required to maintain ionic gradients with reperfusion triggering an inflammatory response which further exacerbates injury. Mechanisms of ischemic injury are common to all solid organs, but there are specific characteristics for each.

One proposed model of ischemia/reperfusion-induced ionic imbalance poises NHE in an important role during both ischemia and reperfusion. NHE is activated by intracellular acidosis upon ischemia (Fig. 4a), but transport is repressed by the low pH of the interstitium. Restoration of flow during reperfusion (Fig. 4b) normalizes first extracellular pH, resulting in maximal kinetic stimulation of NHE. Restoration of intracellular pH by NHE comes at the price of rising intracellular Na⁺ concentration. Normally, the Na⁺/K⁺-ATPase would extrude excess Na⁺, but ATP depletion hampers this process. At high intracellular Na⁺ concentrations, Ca²⁺ extrusion via NCX is inhibited, and NCX can operate in a reverse mode, loading the cell with Ca²⁺ resulting in cellular injury by multiple mechanisms [62]. While activation of NHE restores intracellular pH, the concomitant increase in intracellular Na⁺ can lead to Ca²⁺ overload and exacerbation of tissue injury, a phenomenon termed the pH paradox.

Cardiac ischemia

NHE1 is the predominant isoform expressed in cardiomyocytes, where it functions to rectify intracellular acidification. There is a steep relationship between pH_i and NHE1 activity in cardiac cells, with pK_i of about 7.4 [63]. The relative resistance to cardiac ischemia/reperfusion injury in NHE1^{-/-} mice is comparable with the effect of cariporide inhibition of NHE1 in wild-type mice [22], supporting the significance of NHE1 action in cardiac ischemia/reperfusion injury in the rodent model.

There are two major groups of NHE1 inhibitors: pyrazine derivatives (amiloride, dimethyl amiloride, ethylisopropyl amiloride) and benzoylguanidine derivatives (HOE-694, cariporide, eniporide) [64]. Treatment with both pyrazine and benzoylguanidine derivatives has protective effects in cardiac ischemia/reperfusion injury in animal models – especially if used prior to, rather than during or after ischemia [65-69,70•].

As a warning remark, it has to be pointed out that although pharmacological inhibition is a powerful and valuable tool, its results have to be interpreted and translated to the molecular level with some caution. Amiloride has different inhibitory potencies on different NHEs, but also inhibits epithelial Na⁺ channels, acidsensing ion channels and Na⁺/Ca²⁺ exchangers [64, 71]. Other NHE inhibitors have relatively higher specificities, but are still far from mechanistically inhibiting a single isoform [64]. The same applies for NCX inhibitors [72].

In humans, the GUARDIAN [73] and ESCAMI [74] trials, using cariporide and eniporide respectively, failed to demonstrate a significant benefit in patients with various cardiac ischemic syndromes. However, in patients undergoing coronary artery bypass graft surgery, a subgroup of the GUARDIAN trial, cariporide offered a 25% relative risk reduction of all-cause death or myocardial infarction throughout the 6-month trial [75]. The results of cariporide and eniporide in human trials are surprising and somewhat disappointing considering the wealth of animal data heralding the efficacy of these agents. One possible explanation could be the timing of the treatment. NHE inhibition has been mostly beneficial in animal models if applied

before the onset of ischemia, and has only offered protection in humans undergoing coronary artery bypass graft. Another plausible explanation is that in humans with cardiac ischemia, the effect of NHE inhibitors may be masked by parallel events such as diseased vessels, which are inherently not present in animal models. Studies have started to address this issue by using senescent or atherosclerotic animals, but with inconclusive results as yet [76,77]. While it is difficult to provide treatment preceding ischemia in patients, inhibiting NHE exchange could be beneficial in improving the outcome of ischemia/reperfusion in humans when used in conjunction with other cardioprotective therapies.

Another potential target in the model depicted in Fig. 4 is NCX. In theory, inhibiting NCX should prevent intracellular accumulation of Ca^{2+} while still permitting the myocyte to defend against low pH. In isolated rabbit hearts cariporide infusion before ischemia reduced infarct size, but its infusion upon reperfusion failed to offer protection. In contrast, infusion of the NCX inhibitor KB-R7943 similarly reduced infarct size when infused before and after ischemia, suggesting that ischemia/reperfusion injury is more efficiently suppressed by blockade of NCX than NHE [78]. Similarly, the NCX inhibitor SEA0400 had cardioprotective effects in canine and rodent cardiac ischemia/reperfusion models [79,80]. To date, human trials with NCX inhibitors are not available.

Cerebral ischemia

In neurons, pH_i can affect neuronal excitability by ionchannel gating and, conversely, neuronal activity can alter pH_i . Neuronal activity can vary enormously from one neuron to another; hence production of metabolic acids can vary greatly. Efficient acid-extrusion mechanisms are critical for homeostasis. Because of their high metabolic rate, neurons are susceptible to injury if exposed to excessive activity (seizure) or ischemia (stroke). pH regulation is likely to be crucial in both physiological and pathophysiological conditions. NHE1 plays an important role in the pH regulation of both neurons [81] and astrocytes [82•].

A salient characteristic of cerebral ischemia is the failure of ATP-dependent glutamate transporters to remove glutamate, the major excitatory neurotransmitter, from the synaptic cleft, leading to overactivation of glutamate receptors and a rise in cytosolic Ca^{2+} concentration – a phenomenon termed excitotoxicity [83]. The fact that glutamate receptor antagonists have failed to offer neuroprotection in clinical trials [84] is likely due to the coexistence of multiple mechanisms contributing to ion imbalance in cerebral ischemia. These mechanisms may include opening of Ca^{2+} - and Na^{+} -permeable acid-sensing ion channels (ASIC1a) [85,86], activation of the $\text{Na}^{+}/\text{K}^{+}/\text{Cl}^{-}$ cotransporter NKCC1 [87], excitotoxicity-induced proteolytic cleavage of NCX1 and NCX3 [88], as well as activation of NHE1 [81,82•].

The NHE1 inhibitor SM-20220 significantly reduced the extent of cerebral edema, Na^{+} content and infarcted area in a transient focal ischemia rat model [89]. In primary culture of rat cortical neurons, SM-20220 was protective against glutamate-induced excitotoxicity [90•]. Treatment with cariporide before hypothermic circulatory arrest in pig dramatically improved early neurologic recovery [91]. In primary cultured astrocytes from NHE1^{+/+} mice, oxygen and glucose deprivation led to a 5-fold increase in intracellular Na^{+} and cell swelling. In both NHE1^{-/-} astrocytes and NHE1^{+/+} astrocytes treated with cariporide, the oxygen- and glucose-deprivation-induced Na^{+} rise was less than 2-fold, and swelling was significantly reduced [82•].

Renal ischemia

Five NHE isoforms (NHE1–4 and NHE8) are expressed in the plasma membrane of different renal cells (Fig. 2), rendering the understanding of their individual roles in response to ischemia/reperfusion injury more complex. Rats subjected to renal ischemia/reperfusion

exhibited drastic reduction in kidney NHE3 mRNA, mild decrease in NHE2 and NHE4 mRNA, and mild increase in NHE1 mRNA [92,93]. At the protein level a different study found decreased NHE3, type II Na^+/P_i cotransporter and Na^+/K^+ -ATPase [94]. A pathophysiologic interpretation of these findings could ascribe the observed renal Na^+ wasting to loss of Na^+ transporters due to ischemia damage [92-94]. An alternative and not exclusive view is that this is not a consequence of damage but rather an adaptive mechanism to cope with low ATP supply. If the primary insult is energy limitation, turning off NHE in the proximal tubule can achieve protective effects by blocking apical Na^+ entry and thus reducing Na^+/K^+ -ATPase ATP consumption. The increased distal delivery will repress glomerular filtration rate to avoid massive volume loss as per the 'acute renal success' theory of Thurau and Boylan [95]. Reducing intracellular Na^+ by blocking apical entry may also reduce Ca^{2+} loading by NCX. In one study, the NHE3 inhibitor S3226 improved renal function after experimental ischemia/reperfusion [96]. The NCX inhibitor SEA0400 was beneficial in a rat renal ischemia/reperfusion model [97], and ischemia/reperfusion-induced renal dysfunction was attenuated in NCX1^{+/-} heterozygous mice (having NCX1 protein expression in the kidney decreased to about half) compared to NCX1^{+/+} mice [98].

Conclusion

Vast amounts of published data have started to shed light on the elaborate works of the proteins capable of exchanging Na^+ for H^+ across lipid bilayers. Piecing together this massive body of literature is a formidable task, but even more challenging is the vastness of the areas that remain to be explored. From solving protein structures and explaining transport machineries in molecular detail, to establishing relevance for human disease and therapeutic intervention, Na^+/H^+ -exchange research has yet to reach its full potential.

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Abbreviations

NCX	$\text{Na}^+/\text{Ca}^{2+}$ exchanger
NHE	Na^+/H^+ exchanger
VSMC	vascular smooth muscle cell

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Figure 1. Functions of Na⁺/H⁺ exchangers (NHEs)

NHEs mediate the exchange of Na⁺ for H⁺ across the lipid bilayer. (a) In prokaryotes, the H⁺ gradient is used to energize extrusion of Na⁺ and thus contributes to defense against salinity. In eukaryotes, plasma membrane NHEs utilize the inward Na⁺ gradient to drive H⁺ out, hence fulfilling the role of cell pH defense. When coupled with parallel Cl⁻/base (B⁻) exchange, HB recycling, and water channels, NHEs can restore cell volume when confronted with osmotic cell shrinkage. When positioned in the lipid membrane of organelles, NHEs can regulate organellar pH and/or Na⁺ concentration. Finally, the plasma membrane locale of NHE can serve as a membrane anchor (non-transport function) and assembly site for the cytoskeleton and signaling complexes. (b) In specialized polarized epithelia, apical NHE isoforms (NHE3 and others) mediate several modes of Na⁺-coupled secretion or absorption. Coupling of NHE to H⁺-coupled transporters allows utilization of the Na⁺ gradient and conversion of H⁺-coupled transport to Na⁺-coupled transport. This is the mechanism for renal proximal tubular absorption of some organic solutes by H⁺-coupled cotransport (left absorption model; X stands for oligopeptides, amino acids, etc.) and for the secretion of others by H⁺-coupled countertransport (left secretion model; Z stands for epinephrine, norepinephrine, histamine, amiloride, etc.). NHE can also be coupled with an anion/base (B⁻) exchanger with recycling of HB (center absorption model; Y stands for chloride, urate). In the case of Cl⁻, coupled transport leads to net NaCl transport across the membrane. The extruded H⁺ can also react with a buffer such as HCO₃⁻ which is converted to CO₂. Diffusion of CO₂ across the bilayer results in net NaHCO₃ transport (right absorption model). Finally, NHE can mediate the secretion of ammonium (NH₄⁺), both by providing the H⁺ necessary to trap diffused ammonia (NH₃), and by directly transporting NH₄⁺ as a substrate (right secretion model).

Figure 2. Na^+/H^+ exchanger (NHE) isoforms in the mammalian kidney

NHE isoforms 1–4 and 6–9 are expressed in the kidney. NHE isoforms 1–4 and 8 are in the plasma membrane of renal epithelial cells, with precise localization to the apical or basolateral membrane. The apical isoforms are NHE3 and NHE8 in the proximal tubule, NHE3 and NHE2 in the thick ascending limb of the loop of Henle, and only NHE2 in the distal convoluted tubule and connecting tubule. The thin limbs lack detectable levels of apical NHEs, with the exception of a distinct subpopulation of juxtamedullary nephrons with long loops of Henle, which express low levels of apical NHE3. On the basolateral side, NHE1 and NHE4 are present in all nephron segments, with the exception of the macula densa and intercalated cells of the cortical collecting duct, where NHE4 is the only basolateral isoform. NHE isoforms 6, 7 and 9 are only in organellar membranes (not shown) [5•,6•,7•,8-10].

Figure 3. Theoretical links between Na^+/H^+ exchange overactivation and hypertension

Overactivation of Na^+/H^+ exchanger (NHE) 3 in the kidney may lead to salt and fluid retention, increasing the effective circulatory volume. Independently or concurrently, overactivation of NHE1 in vascular smooth muscle cells (VSMCs) may lead to intracellular Na^+ loading, resulting in slowing or reversal of $\text{Na}^+/\text{Ca}^{2+}$ exchange via $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX), increased cytosolic Ca^{2+} and VSMC contraction. At the same time, NHE1 activity may promote VSMC growth and proliferation, the overall result being increased peripheral resistance.

Figure 4. Theoretical model of Na^+/H^+ exchange in ischemia/reperfusion

(a) Ischemia leads to intracellular acidosis by anaerobic glycolysis and mitochondrial dysfunction. Na^+/H^+ exchange is driven by the intracellular/extracellular H^+ gradient and allosterically activated by intracellular protons (dashed curved arrow). Acidification of the interstitium (low pH_e) partially suppresses the gradient-driven Na^+/H^+ exchange. At the same time, cellular ATP depletion leads to Na^+/K^+ -ATPase dysfunction. The net result is intracellular sodium loading. Rising $[\text{Na}^+]_i$ slows down the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX), impairing calcium extrusion from the cell. (b) During reperfusion pH_e normalizes and Na^+/H^+ exchange is fully activated by allosteric regulation and ionic gradients. With cellular ATP and Na^+/K^+ -ATPase activity still insufficient, intracellular Na^+ rises to yet higher levels. At some point during this process, rising $[\text{Na}^+]_i$ leads to operation of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger in reverse mode. NCX extrudes Na^+ at the price of raising $[\text{Ca}^{2+}]_i$, which can achieve deleterious levels and lead to cell injury and apoptosis. NHE, Na^+/H^+ exchanger.

Table 1

Mammalian Na⁺/H⁺ exchanger (NHE) family

Protein	Human gene	Human gene locus	Human GeneID (NCBI)	Tissue expression	Cellular distribution
NHE1	SLC9A1	1p36.1–35	6548	Ubiquitous, with some exceptions such as the macula densa and intercalated cells of the cortical collecting duct	Plasma membrane; basolateral membrane of epithelial cells
NHE2	SLC9A2	2q11.2	6549	Gastrointestinal tract, skeletal muscle; less in kidney, brain, testis and uterus	Plasma membrane; apical membrane of epithelial cells
NHE3	SLC9A3	5p15.3	6550	Gastrointestinal tract, kidney and gall bladder; epididymis; much less in brain	Plasma membrane; apical membrane and recycling endosomes of epithelial cells
NHE4	SLC9A4	2q12.1	389015	Stomach; less in kidney and brain	Plasma membrane; basolateral membrane of epithelial cells
NHE5	SLC9A5	16q22.1	6553	Brain	Plasma membrane and recycling endosomes (synaptic vesicles)
NHE6	SLC9A6	Xq26.3	10479	Ubiquitous	Intracellular (recycling endosomes)
NHE7	SLC9A7	Xp11.3–11.23	84679	Ubiquitous	Intracellular (<i>trans</i> -Golgi network)
NHE8	SLC9A8	20q13.13	23315	Ubiquitous; luminal surface of renal and intestinal epithelia	Intracellular (<i>trans</i> -Golgi network); apical membrane and recycling endosomes of epithelial cells
NHE9	SLC9A9	3q24	285195	Ubiquitous	Intracellular (recycling endosomes)
Sperm NHE	SLC9A10	3q13.2	285335	Spermatozoa	Sperm flagellum

NCBI, National Center for Biotechnology Information, US National Library of Medicine.

Table 2

Na⁺/H⁺ exchanger (NHE)-defective mouse models

Model	Genotype	Phenotype
<i>swe</i> mouse (slow-wave epilepsy, spontaneous mutant)	SLC9A1 ^{swe/swe} nonsense mutation: Lys-442	Growth retardation, seizures (petit mal and grand mal), locomotor ataxia, high mortality before weaning [20]
NHE1 ^{-/-} (knockout)	SLC9A1 ^{-/-}	Same overall phenotype as <i>swe</i> mouse [21]; decreased parotid gland secretion [14]; resistance to experimental cardiac ischemia/reperfusion injury [22]; decreased renal HCO ₃ ⁻ absorption [18•]; altered (mostly downregulated) expression of multiple genes in the brain [23]
NHE2 ^{-/-} (knockout)	SLC9A2 ^{-/-}	No overt disease phenotype, no renal or intestinal absorptive defects; mild degeneration of gastric parietal and zymogenic cells [24]; decreased parotid gland secretion [14]
NHE3 ^{-/-} (knockout)	SLC9A3 ^{-/-}	Hypovolemia, hypotension, mild diarrhea, mild metabolic acidosis, decreased renal absorption of Na ⁺ , fluid and HCO ₃ ⁻ , proteinuria, increased mortality when fed a low-salt diet [25]; reduced steady-state glomerular filtration rate (evaluated in both whole kidney and single nephron), likely due to shifted tubulo-glomerular feedback sensitivity [26]; blunted flow-stimulated increase in fluid absorption in the proximal tubule (normally mediated through microvillar mechanosensing and transmitted to NHE3 by the actin cytoskeleton) [27••]
NHE3 ^{-/-} (knockout) with small intestine rescue	Tg(IFABP-NHE3) SLC9A3 ^{-/-}	Improved compared to the NHE3 knockout (higher blood pressure, better tolerance to low-salt diet), but remains hypovolemic and hypotensive on normal-salt diet [28,29•]
NHE2 ^{-/-} NHE3 ^{-/-} (double knockout)	SLC9A2 ^{-/-} SLC9A3 ^{-/-}	Same as NHE3 knockout, with no further worsening of acidosis, hypovolemia, diarrhea or proximal tubule NHE activity [30,31]
NHE4 ^{-/-} (knockout)	SLC9A4 ^{-/-}	No overt disease phenotype; slightly impaired gastric acid secretion and histological abnormalities of the gastric mucosa [32]; renal function not studied extensively
Sperm- NHE ^{-/-} (knockout)	SLC9A10 ^{-/-}	Impaired sperm motility, male infertility [33]

IFABP, intestinal fatty acid-binding protein promoter.

Table 3
Proximal tubular Na^+/H^+ exchange in hypertension rat models

Hypertensive model vs normotensive control	NHE3 mRNA	NHE3 protein	Na^+/H^+ -exchange activity	Methods	Reference
Spontaneously hypertensive (SHR) vs Wistar–Kyoto (WKY)	ND	ND	Increased	e	[51]
	Not altered	ND	Increased	a, g	[52]
	Increased	Increased	Increased	a, d, g	[53]
	Not altered	Increased	Increased	a, d, f, g	[54]
	Not altered	ND	Increased	b, h	[55]
Milan hypertensive (MHS) vs Milan normotensive (MNS)	ND	ND	Not altered	i	[56]
	ND	ND	Increased	e	[57]
Dahl salt-sensitive on high-salt vs low-salt diet	Not altered	Not altered	ND	c, d	[58•]
	Decreased	ND	Decreased	b, h	[55]
Dahl salt-sensitive vs salt-resistant Brown–Norway, on high- or low-salt diet	ND	Not altered	ND	d	[59]
Unilateral renal cortical injection of phenol vs saline in Sprague–Dawley rats (model of neurogenic hypertension)	ND	Decreased	ND	d	[60]

Methods: a, RNA blot; b, ribonuclease-protection assay; c, real-time competitive PCR; d, immunoblot; e, $^{22}\text{Na}^+$ uptake in brush-border membrane vesicles; f, Na^+ -dependent Acridine Orange fluorescence recovery after quenching in brush-border membrane vesicles; g, fluorimetric assay of Na^+ -dependent pH_i recovery in suspended proximal tubules; h, fluorimetric assay of Na^+ -dependent pH_i recovery in primary culture of proximal tubular cells; i, $^{22}\text{Na}^+$ uptake in primary culture of proximal tubule cells. ND, not determined; NHE3, Na^+/H^+ exchanger isoform 3.