Deciphering the mechanisms of the Na\(^+\)/H\(^+\) exchanger-3 regulation in organ dysfunction

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Girardi AC, Di Sole F. Deciphering the mechanisms of the Na\(^+\)/H\(^+\) exchanger-3 regulation in organ dysfunction. Am J Physiol Cell Physiol 302: C1569–C1587, 2012. First published March 28, 2012; doi:10.1152/ajpcell.00017.2012.—The Na\(^+\)/H\(^+\) exchanger-3 (NHE3) belongs to the mammalian NHE protein family and catalyzes the electro-neutral exchange of extracellular sodium for intracellular proton across cellular membranes. Its transport function is of essential importance for the maintenance of the body’s salt and water homeostasis as well as acid-base balance. Indeed, NHE3 activity is finely regulated by a variety of stimuli, both acutely and chronically, and its transport function is fundamental for a multiplicity of severe and world-wide infection-pathological conditions. This review aims to provide a concise overview of NHE3 physiology and discusses the role of NHE3 in clinical conditions of prominent importance, specifically in hypertension, diabetic nephropathy, heart failure, acute kidney injury, and diarrhea. Study of NHE3 function in models of these diseases has contributed to the deciphering of mechanisms that control the delicate ion balance disrupted in these disorders. The majority of the findings indicate that NHE3 transport function is activated before the onset of hypertension and inhibited thereafter; NHE3 transport function is also upregulated in diabetic nephropathy and heart failure, while it is reported to be downregulated in acute kidney injury and in diarrhea. The molecular mechanisms activated during these pathological conditions to regulate NHE3 transport function are examined with the aim of linking NHE3 dysfunction to the analyzed clinical disorders.

hypothesis; diarrhea; acute kidney injury; diabetic nephropathy; heart failure

THE REGULATED MOVEMENT of ions across biological membranes affects a variety of cellular processes that are of essential importance for all living organisms. A variety of ion channels, pumps, and transporters accomplishes these fundamental functions. For instance, cells routinely expel sodium (Na\(^+\)) ions via the Na\(^+\)-K\(^+\)-ATPase to create and maintain a lower concentration of Na\(^+\) in the cytosol than in the surrounding extracellular fluid which generates the electrochemical gradient that drives most Na\(^+\)-coupled transport processes.

The Na\(^+\)/H\(^+\) exchangers (NHEs) are typical Na\(^+\)-coupled transporters that mediate the counter-transport of one extracellular Na\(^+\) for one cytosolic proton (H\(^+\)) across the lipid bilayers (20, 21, 157, 189). NHEs are found ubiquitously in prokaryotes, lower and higher eukaryotes. In mammals the NHE family consists of nine related gene products (NHE1-9) and a cluster of distant NHE-related genes (NHA1 and NHA2) (157). The human genome also contains a sperm-specific NHE (211).

The members of the mammalian NHE protein family can be broadly classified depending on their cellular localization.

NHE1 through NHE5 are targeted to the plasma membrane, though NHE3 and NHE5 isoforms can also enter endosomal pools (20, 157). NHE6 to NHE9 are thought to reside mainly in the endomembrane compartment, with the exception of NHE8 mostly intracellularly located in adults but highly expressed at the plasma membrane in neonates (20, 21, 157). Importantly, localization of each isoform predominantly correlates with its key function. Hence, members of the mammalian NHE family regulate a myriad of essential physiological functions, which have been extensively summarized (20, 21, 157, 189, 231). This review specifically highlights the molecular basis for regulation of isoform 3, NHE3, in certain pathophysiologic conditions.

NHE3 has a limited tissue distribution and is confined mainly to the apical surface of renal and gastrointestinal epithelial cells (6, 231). NHE3 localization correlates with its function in the direct reabsorption of filtered sodium along with indirect reabsorption of bicarbonate and chloride and secretion of ammonium. NHE3 activity also contributes to the reabsorption of filtered amino acids, oligopeptides, proteins, and citrate (reviewed in Refs. 20, 21, 231). Furthermore, NHE3 is located at the plasma membrane and in the endosomal compartment and it is believed to function in both locations. It has been postulated that intracellular NHE3 is important for endosomal
acidification particularly in the filtered proteins reabsorption by receptor-mediated endocytosis (65). In view of the multiple functions of NHE3, it is not surprising that NHE3 is regulated by a large variety of agonists and physiological conditions (reviewed in Refs. 20, 21, 231). After two decades since the identification of NHE3 as the main luminal renal and gastrointestinal NHE isofrom, open questions remain about the molecular mechanisms of acute and chronic NHE3 regulation. To date, NHE3 activity has been found to be acutely regulated by several mechanisms such as modifications in the rate of transport, trafficking, and NHE3 association with partner proteins. These layers of acute control reflect the need for rapid, transient, and often reversible regulatory events to respond promptly to physiological challenges (reviewed in Refs. 20, 21, 231).

NHE3 is also regulated by chronic changes in protein abundance via transcriptional and posttranscriptional or proteosomal regulation. These mechanisms are believed to reflect rather a slower and more permanent regulation which provides the basis for long-term adaptation and are of particular significance for the pathophysiology of disease (reviewed in Refs. 20, 21, 231). Yet, there is no definitive classification of the mechanisms of NHE3 regulation activated in clinical conditions. The challenge in the study of the mechanisms of NHE3 regulation in organ dysfunction is that much of the current knowledge on NHE3 regulation is based on heterologous expression of NHE3 in fibroblast-like cells or endogenous expression in cultured-renal and -intestinal epithelial cells. Although these studies have been critical for the progress of the field, their recent confirmation using animal models has in some cases yielded unexpected or contradictory findings. The following sections discuss NHE3 regulation in the contest of clinical disorders of prominent severity and incidence, including hypertension, diabetic nephropathy, heart failure, acute kidney injury, and diarrhea. The discussion includes detailed features of the regulatory mechanisms as well as the implications of NHE3 regulation.

Differential Regulation of NHE3 Activity Before and After Development of Hypertension

Hypertension is a disease characterized by chronic elevation of blood pressure; it affects 20 to 30% of the world population (106) and represents the major risk factor for coronary heart disease, cerebrovascular disease, and kidney failure. The recent study by Lawes et al. (124) concluded that high blood pressure is associated with 54% of strokes, 13.5% of premature deaths, and 47% of ischemic heart disease. Nevertheless, most cases of hypertension have no identifiable cause and are classified as essential hypertension.

Essential hypertension is a complex and multifactorial disease, i.e., different factors may contribute to its development (50). A compelling body of clinical and experimental evidences documents the importance of the kidney in the pathogenesis and maintenance of arterial hypertension. Experiments conducted in several hereditary strains of hypertensive rats demonstrate that the donor’s hypertension is transplanted, along with the kidney, to a normotensive strain recipient that has been nephrectomized and, vice versa, that a normotensive kidney can correct or attenuate hypertension in a nephrectomized hypertensive animal (48, 86, 119, 170, 171). Similar observations have been documented in humans: in a classic study Curtis and colleagues (47) studied individuals who received transplanted kidneys from normotensive donors after their own kidneys had failed due to “essential hypertension” and nephrosclerosis and found that more than 4 years later they had normal blood pressure and reversal of the hypertensive damage to their heart and retinal vessels. These findings support the notion that the kidneys contain the blood pressure set-point and that “hypertension travels with the kidney”; thus, defects in renal function may determine the level of arterial hypertension.

Essential hypertension is characterized by a disturbance of renal function that increases Na+ reabsorption and decreases Na+ output below Na+ intake (39, 44, 76). Thus, there is an accumulation of Na+ in the body, promoting a marked expansion of extracellular volume and therefore cardiac output. As cardiac output increases, it raises the blood flow to virtually all body tissues. In response, an autoregulatory mechanism for local control of blood flow causes an immediate adjustment in the blood vessel diameter, reestablishing adequate tissue perfusion. Autoregulation therefore increases peripheral vascular resistance and blood pressure. The price for this biological adaptation is hypertension. Hypertension provides the error signal for reestablishing extracellular fluid volume by turning on pressure natriuresis.

Changes in renal function that may result in the loss of the kidneys’ ability of maintaining Na+ balance under normal blood pressure levels include decreased glomerular filtration rate (GFR), defects in tubular transport of Na+ and water, or both. During the past few decades, attempts to define the molecular determinants of hypertension by association and positional cloning identified close to 20 genes related to high or low blood pressure (131), including rare Mendelian diseases and essential hypertension. Interestingly, many of these genes encode proteins that mediate or are involved in control of renal tubular Na+ handling, namely, ion channels, membrane transporters, or components of signaling pathways that regulate their activities.

Gary Shull’s laboratory generated mice with homozygous disruption of the NHE3 gene, thereby knocking out NHE3 function (184). The key role of NHE3 on extracellular volume homeostasis and long-term control of blood pressure is underscored by the phenotype of NHE3 null mice. These animals have sharp reduction of the rate of fluid reabsorption in the proximal tubule and exhibit several characteristic of chronic volume depletion including hypovolemia, hypertension, higher serum aldosterone as well as higher mRNA encoding renal renin (184). The major compensatory mechanism to prevent renal salt loss in NHE3 null mice is a decrease in GFR mediated by tubuloglomerular feedback (135). It is noteworthy to underline that NHE3 is highly expressed in the small intestine as well as the kidney and that the overall effect of NHE3 on extracellular fluid volume and blood pressure is also mediated by the intestinal defect that leads to lower absorption of salt and water (218). In an attempt to define the role of renal NHE3 in systemic maintenance of blood pressure and pressure natriuresis, Woo et al. (218) generated NHE3 null mice with transgenic expression of NHE3 in the small intestine (tgNhe3−/−). They observed that the animals present some degree of improvement in their chronic volume depleted state and tolerated better variations in the dietary Na+ intake compared with
The indicated clinical conditions. Up arrow (↑) represents an increase of the specific modification; down arrow (↓) represents a decrease of the specific modification. ND, not determined. Reference numbers are shown in parentheses.

Table 1. Summary of the reviewed data divided for levels of NHE3 regulation

<table>
<thead>
<tr>
<th>Clinical Condition</th>
<th>NHE3 Activity</th>
<th>NHE3 Protein</th>
<th>NHE3 Trafficking</th>
<th>NHE3 mRNA</th>
</tr>
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<tbody>
<tr>
<td>Hypertension</td>
<td>↑ In young (prehypertensive) rats (45, 82, 109, 115, 122, 201)</td>
<td>No change in total (27, 28, 45, 82, 138); ↑ in total (109, 122)</td>
<td>From the body to the base of the microvilli (138, 223, 224, 227)</td>
<td>No change (27, 45, 82, 115, 122); ↑ (109)</td>
</tr>
<tr>
<td></td>
<td>↓ In adult (hypertensive) rats (45, 160, 201)</td>
<td>In young (prehypertensive) rats the ratio between phosphorylated and total NHE3 is decreased and in adult (hypertensive) rats it is increased (45).</td>
<td>In young (prehypertensive) rats from the base to the body of microvilli (45)</td>
<td></td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>↑ (79, 113, 164, 209)</td>
<td>↑ In total (5, 58, 181, 191); no change in total (113)</td>
<td>Reinsertion in cell culture model (114)</td>
<td>↑ (5, 58, 181); No change (113); ↑ (100)</td>
</tr>
<tr>
<td>Heart failure</td>
<td>↑ (100)</td>
<td>↑ In total (100, 137)</td>
<td>From the base to the body of the microvilli (100)</td>
<td>↑ (100)</td>
</tr>
<tr>
<td>Acute kidney injury</td>
<td>ND</td>
<td></td>
<td>ND</td>
<td>↓ (51, 215, 216)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>↓ (81, 85, 93, 132, 226)</td>
<td>↓ In surface (81) and total (196, 197); no change in total (81, 226)</td>
<td>Internalized in cell culture model (81)</td>
<td>No change (196, 226)</td>
</tr>
</tbody>
</table>
of these results, Noonan et al. (152) demonstrated that the \( \text{tgNhe3}^{-/-} \) lineage that lacks renal NHE3 activity displays a blunted pressure-natriuresis response at low renal perfusion pressures (Fig. 1A).

Crajoinas et al. (45) have recently demonstrated that the differential regulation of NHE3 function before and after development of hypertension in SHR is associated with changes in the endogenous phosphorylation of the transporter at PKA consensus site serine 552. As such, in young prehypertensive SHR the ratio of phosphorylated NHE3 to total NHE3 (P-NHE3/total) in renal cortical membranes is significantly lower compared with WKY (~70%) and correlates with the degree of NHE3 transport stimulation. Likewise, the P-NHE3/total ratio is increased in SHR (~70%) and correlates with the reduction of NHE3 activity (~60%). It is therefore suggestive that the differential regulation of NHE3 transport function before and after development of hypertension in SHR might be due to abnormalities that disturb the balance between the activities of kinases and phosphatases in the renal proximal tubule.

Fig. 1. Model of how the Na\(^+/H^+\) exchanger-3 (NHE3) participates in the pressure-natriuresis response and how its transport function is decreased in hypertension. A: acute and chronic increases in blood pressure cause changes in renal perfusion pressure (RPP) that are sensed by the kidneys. In response to these changes, the production of intrarenal signaling molecules such as NO (103) and metabolites of the cytochrome P450 (53, 232) increases. Some of these signaling molecules induce NHE3 to move down from the body to the base of the microvilli (MV). Whether some of these factors are capable of inducing phosphorylation at the PKA consensus site (RRX/SLY) on the COOH terminus of NHE3, at serine 552, and whether phosphorylation of NHE3 at this residue is necessary to induce changes on NHE3 redistribution are yet to be defined. Distribution of NHE3 from the body to the base of the MV decreases NHE3-mediated sodium reabsorption in the proximal tubule (PT) and increases sodium excretion. In view of the fact that NHE3 subcellular distribution cannot serve as a natriuretic mechanism in the NHE3 null mice with transgenic expression of NHE3 in the small intestine (tgNhe3\(^{-/-}\)), the pressure-natriuresis response is blunted in these animals (152). B: NHE3 exists in physical complexes with dipeptidyl peptidase IV (DPPIV) in MV membranes, and transport studies have shown that DPPIV plays a tonic role in modulating NHE3-mediated sodium reabsorption in the proximal tubule. The interaction between NHE3 and DPPIV is not direct and requires additional intermediary partners that remain to be identified. C: acute and chronic hypertension provoke redistribution of NHE3 along with its regulator DPPIV from the body to the base of the microvilli which may be dependent on increases of NHE3 phosphorylation. When NHE3 is phosphorylated at serine 552 it predominantly localizes in the base of the microvilli where NHE3-mediated sodium reabsorption is postulated to be inhibited. The molecular motor myosin VI may mediate the dynamic subcellular trafficking of the NHE3-DPPIV complex triggered by hypertension as an integral part of the pressure natriuresis response. This model requires further experimental validation.
It has been demonstrated that dipeptidyl peptidase IV (DPP-IV) coordinately redistributes with NHE3 between the microdomains of the kidney brush border with the development of hypertension in the SHR (45, 138). Girardi and colleagues reported that NHE3 and DPPIV reside together as an oligomeric complex in the renal proximal tubule (66) and that inhibition of DPPIV downregulates NHE3 activity (67, 68). Furthermore, DPPIV inhibition has been shown to attenuate blood pressure rising in young prehypertensive SHR, at least in part, by reducing NHE3 activity (158). These as well as other recent findings suggest the therapeutic potential of DPPIV inhibitors as antihypertensive agents (198).

The association between NHE3 and DPPIV is not direct and requires the presence of additional accessory proteins (69). It is tempting to speculate that one of these accessory proteins mediates the dynamic subcellular trafficking of the NHE3-DPPIV complex in response to physiological, pathological, and pharmacological stimuli (Fig. 1, A and C). In this context, Yang and colleagues (225) observed that the cytoskeleton protein myosin VI also retracts out of the microvilli in response to acute blood pressure. Myosin VI is an unconventional myosin that moves toward the pointed end of actin filaments located at the base of the brush border microvilli. On the basis of these observations, one may conclude that myosin VI is a potential candidate to move NHE3 and DPPIV out of the microvilli in response to elevation of blood pressure (Fig. 1C).

It remains to be determined whether myosin VI specifically associates with NHE3 and/or DPPIV in the renal proximal tubule, but in support of this idea, all three of these proteins preferentially distribute to ordered lipid domains in the proximal tubule, distinct from other microvillar proteins such as the Na-phosphate cotransporter and its accessory protein NHERF1 (sodium proton exchanger regulatory cofactor-1) which distribute to nonordered membrane domains (175).

The important role of NHE3 in the control of extracellular volume and blood pressure raises the possibility that mutations in the Nhe3 gene could be involved in human essential hypertension. A study by Zhu et al. (233) was specifically designed to test this hypothesis. These investigators screened eight exons within the COOH region of Nhe3 in 200 subjects (100 of Caucasian origin and 100 of African or African-Caribbean origin) and identified six variants. Four of them were silent polymorphisms, one was relatively rare, and the other, located in a potential calmodulin-binding site, was found not to be associated with high blood pressure. The authors chose to evaluate exons within the COOH-terminal portion of the NHE3 based on the fact that the members of the NHE family are targeted and regulated in an isoform-specific way and that this specific responsiveness is mainly provided by the COOH-terminal domain (32). However, it is possible that genetic variants may exist in other regions of the Nhe3 gene, especially in the promoter region. In any event, the lack of association between Nhe3 variants and blood pressure does not rule out the potential importance that this exchanger plays in the pathophysiology of essential hypertension. If fact, the interpretation of the data reviewed here favors the conclusion that relevant mechanisms implicated in both development and maintenance of hypertension may be unraveled through the elucidation of signaling pathways that control NHE3 abundance and phosphorylation as well as that of its binding partners that mediate subcellular trafficking. For example, a recent study in mice found that a specific deletion of the angiotensin II receptor (AT1) from the proximal tubule alone resulted in reduced blood pressure along with reduced NHE3 and fluid reabsorption and improved pressure natriuresis response (75).

Role of NHE3 in the Pathophysiology of the Diabetic Kidney

Diabetes mellitus is a chronic health condition in which the body either fails to produce sufficient amounts of insulin or responds abnormally to insulin. It affects 25.8 million of the world population and 8.3% of the United States population (2). Furthermore, diabetes mellitus is accompanied by many long-term complications; prominent among these is the diabetic nephropathy (40, 99).

Diabetic kidney disease is characterized by the appearance of protein in the urine (persistent microalbuminuria), elevated arterial blood pressure (hypertension), and persistent decline in GFR. The urinary microalbuminuria is due to the increased leak at the glomerulus and also to the decreased reabsorption of albumin by the renal proximal tubule. The hypertension is mediated by the systemic volume expansion due to retention of salt and water. In particular, the early phase of diabetic nephropathy is characterized by changes in glomerular filtration, increased tubular salt and water reabsorption, and expansion of systemic volume, which may all contribute to the progression of hypertension, continued hyperfiltration, and renal hypertrophy (208, 209).

Approximately 20% of patients with either type 1 (insulin-dependent) or type 2 (insulin-independent) diabetes mellitus have evident nephropathy (207). Although only a subset of diabetic patients is susceptible to renal damage, the factors (e.g., genes, proteins) that confer the susceptibility to or protection from renal disease of diabetic patients remain unknown. Identification of these factors is relevant for the determination of the molecular mechanisms leading to diabetic nephropathy and documentation of mediators useful for early diagnosis. Indeed, understanding the factors that regulate renal Na+ handling in diabetes provides insight into the molecular pathways that contribute to the progression and deterioration of renal function in diabetic patients.

An interesting observation in this regard is that diabetic kidneys are larger than normal and grow from the onset of the disease. Elongation of the proximal tubules was found to be responsible in greatest proportion for this growth (reviewed in Ref. 209). The total length of proximal tubules in streptozotocin (STZ)-diabetic rats increased up to 22% when compared with normal proximal tubules (168). The STZ-diabetic rat is a suitable model for testing therapeutic interventions for improvement of chronic diabetic complications in humans. Indeed, this diabetic animal model mimics many (but not all) of the chronic complications associated with diabetes. STZ treatment is cytotoxic for the pancreatic insulin-producing β-cells. Uptake of STZ by the β-cells via the facilitative glucose transporter (GLUT2) induces DNA fragmentation and alkylatation followed by cell apoptosis (57, 146, 221). In this animal model, the increase in length of tubules could simply result in an increase in proximal tubule reabsorption (209) by activation of Na+-transporter activity. NHE3 is responsible for the bulk of salt reabsorption in the proximal tubule, and its activity may be affected the most by the increase in length of this renal segment. Indeed, it has been demonstrated that NHE3 activity
is increased in rats with STZ-induced diabetes (79, 164, 209). After 7 and 14 days of STZ treatment, brush border membrane NHE3 activity was increased ~40%, without elevation in NHE3 protein or transcript expression (113). Song et al. (191), using a similar animal model, confirmed these findings and also reported an increase of NHE3 total protein assayed in the whole homogenate. These data indicate an upregulation of NHE3 function in STZ-diabetic rats; they suggest, but do not prove, a correlation between the augmented activity of NHE3 and the increase in length of the proximal tubules in this animal model of diabetic nephropathy. Indeed, in the early stage of experimental and clinical diabetes, there is a significant increase in GFR (hyperfiltration). Therefore, the larger proximal tubule and increased sodium absorption via activation of NHE3 activity may be simply the result of maintaining a balanced glomerular-proximal tubule response. However, enhanced tubular sodium reabsorption may be a factor contributing to the hyperfiltration phase, raising the question of whether the increase in GFR in the early stage of diabetes is the “cause” or the “effect.”

The increase in NHE3 activity in diabetic kidneys was further confirmed in several studies using cell culture models. Ambuhl and colleagues demonstrated in opossum kidney (OK) cells, a well-characterized proximal tubule cell line, that treatment with high glucose (to mimic hyperglycemia) results in stimulation of NHE3 activity accompanied by increases in both NHE3 protein and transcript expression (5). Similar results were found in human proximal tubular cells (hPTC) exposed to high glucose (181). High glucose treatment also leads to an increase in expression of the serum glucocorticoid-regulated kinase-1 (SGK1) in hPTC (181); activation of Sgk1 gene expression was also confirmed in STZ-diabetic rats (181). In summary, activation of SGK1 is a good candidate signaling pathway to regulate NHE3 activity in diabetic nephropathy. In support of this notion, synthetic thiazolidinedione derivative ligands of peroxisome proliferator-activated receptor-γ (PPARγ), the activation of which has been proven clinically to improve insulin sensitivity and lower blood glucose level in diabetic patients (72), stimulate SGK1 expression in hPTC (180). PPARγ activation increases NHE3 protein expression in the same cell model, and knockdown of SGK1 by specific small interfering RNA reversed the PPARγ-mediated activation of NHE3.

Interestingly, the expression of other members of the SGK family is altered during diabetes. Similar to SGK1, SGK2 and SGK3 transcripts’ expression levels were elevated in renal cortex of STZ-diabetic mice compared with the control group (214). Furthermore, SGK2 activation was found to regulate NHE3. The expression of the constitutively active SGK2 (SGK2-S356D, an SGK2 with an aspartate-to-serine mutation at position 356, pseudo-phosphorylated SGK2) in OK cells resulted in stimulation of NHE3 activity and in an increase in its cell surface expression (161). However, in the same study, expression of the constitutively active SGK1 (SGK1-S422D, an SGK1 with an aspartate-to-serine mutation at position 422, pseudo-phosphorylated SGK1) did not increase NHE3 activity (161). The apparent discrepancy between these studies (161, 180, 181) may be due to the use of different cell systems. Indeed, OK cells were found to express a low level of the Na+/H+ exchanger regulatory cofactor-2 (NHERF2) (229), which tethers NHE3 and SGK1 to facilitate phosphorylation of NHE3 by SGK1 (212, 213, 229).

The SGK family seems to be a key signal intermediate in the regulation of NHE3 activity and expression in diabetic nephropathy. However, the identity of upstream signaling to SGK activation is unclear.

Administration of angiotensin-converting enzyme inhibitors and angiotensin receptors blockers reduces progression of diabetic nephropathy (128, 129, 209). These findings indicate that the antinatriuretic angiotensin II and its action to increase extracellular fluid volume by stimulating Na+ reabsorption and to increase vascular resistance (25) may be fundamental for the etiology of this pathology. Support of this view came from several studies. High levels of glucose were found to stimulate the expression of angiotensinogen (21), the angiotensin II precursor (25), as well as trigger renin release, critical for processing of angiotensinogen to angiotensin II (162). High glucose treatment also increases angiotensin II type 1 (AT1) receptor transcript expression in hPTC (195); the AT1 receptor is considered the angiotensin II receptor subtype responsible for the overall action of angiotensin II on renal hemodynamic and tubular function (80). Furthermore, oxidative stress, which has been associated with the development and progression of diabetic nephropathy (208), induced by treatment with the oxidant L-buthioninesulfoximine, also increased the expression of AT1 receptors in rats (11).

Since it is well documented that angiotensin II stimulates NHE3 transport function and redistributes it into the microvilli (11, 61, 133, 134, 174, 182), local increase in the level of angiotensin II due to the high glucose stimulation of angiotensinogen expression, to renin secretion, and to an increase in AT1 receptor expression during diabetic nephropathy may result in stimulation of NHE3 activity and in augmentation of salt retention.

Interestingly, it was found that angiotensin II treatment leads to an increase in SGK1 protein and transcript expression. The augmented SGK1 expression resulted in stimulation of NHE3 activity and induction of NHE3 protein and transcript expression (195). The effect of angiotensin II on NHE3 was enhanced by treatment with high glucose and reversed by SGK1 knockdown (195). Overall, these studies suggested coordinated stimulation of NHE3 by angiotensin II and SGK1 to increase salt and water reabsorption during diabetic nephropathy.

However, SGK1 activation by angiotensin II to regulate NHE3 may not be the only signal pathway downstream to AT1 receptor activation. c-Src (a nonreceptor tyrosine kinase), protein kinase C (PKC) and phosphatidylinositol 3-kinase activation have all been described as signal intermediates in the angiotensin II-mediated upregulation of NHE3 (55, 95, 206). Recently, novel signal cascades in angiotensin II-induced stimulation of NHE3 were identified. Study in OK cells revealed that activation of AT1 receptors results in an increase in intracellular calcium (Ca2+) and subsequent activation of Ca2+/calmodulin-dependent protein kinase II (CaMKII) (83). Active CaMKII phosphorylates IRBIT (inositol 1,4,5-triphosphate receptor-binding protein released with inositol 1,4,5-triphosphate), which binds to and induces NHE3 trafficking to apical membrane (83). The importance of an increase in intracellular Ca2+ in angiotensin II-induced stimulation of NHE3 was also shown in rats; AT1 receptor activation induced the formation of the protein complex between the Ca2+.
calmodulin and Janus kinase 2 and stimulation of NHE3 activity (12).

In summary, high glucose concentration and oxidative stress both stimulate NHE3 activity via angiotensin II receptor activation. Multiple signal pathways are activated by angiotensin II to regulate NHE3. The main signaling pathway downstream from angiotensin II activation of AT1 receptor that stimulates NHE3 activity during diabetic nephropathy remains to be determined.

Progression of diabetic nephropathy correlates with persistent microalbuminuria along with rise in arterial blood pressure and the persistent decline in GFR. Development of microalbuminuria in diabetic nephropathy is associated with damage of the glomerular filtration barrier (101). However, there is clear evidence that in diabetic nephropathy the reabsorption in the renal proximal tubule of the filtered albumin is also impaired (42). Uptake of albumin by the proximal tubules from glomerular filtrate occurs via receptor-mediated endocytosis, which requires the formation of the megalin/cubulin receptor complex and several accessory plasma membrane transport proteins (36, 97). In STZ-diabetic rats the tubular reabsorption of albumin is decreased. This drop in albumin reabsorption seems to be associated with a reduction in megalin expression in the proximal tubules (203) and with an increase in the level of transforming growth factor β1 (TGF-β1) (178), TGF-β1 that per se decreases albumin endocytosis in OK cells (62). TGF-β1 upregulation appears predominantly in all forms of chronic kidney disease and in the epithelial-to-mesenchymal transition in diabetic nephropathy (91).

Furthermore, in a study using OK cells, it was shown that NHE3 is one of the plasma membrane proteins necessary for albumin endocytosis in the proximal tubule (64); indeed, NHE3 association with megalin was found in rabbit proximal tubules (16). High glucose treatment of OK cells increases albumin uptake (54) and stimulates NHE3 function and protein expression (5), supporting a further link between NHE3 transport function and albumin endocytosis. Moreover, exposure of OK cells to pathophysiological concentrations of albumin reduces albumin endocytosis (63), though, unexpectedly, it stimulates NHE3 activity and protein expression (114, 126) by increasing the rate of reinsertion of endocytosed NHE3 (114).

In summary, the current body of data on the role of NHE3 in albumin endocytosis seems contradictory. On the one hand, a condition that mimics diabetic nephropathy stimulates activity and expression of NHE3, while on the other hand, the same condition inhibits albumin endocytosis. However, the present findings may be explained by the hypothesis that NHE3 exists at least in two pools; one pool associates with megalin and the other pool is not associated with megalin (16) (Fig. 2A). Possibly, these NHE3 pools are also spatially distributed: the

Fig. 2. Model of how NHE3 could participate in the reabsorption of filtered proteins and how its transport function could be increased in diabetic nephropathy. A: under physiological conditions, NHE3 interacts with megalin, which is responsible for receptor-mediated endocytosis of low-molecular-weight proteins such as albumin. NHE3 may be recruited in endocytic vesicles in complex with megalin and promote albumin uptake. The complex may be degraded in the lysosomes. B: the proteinuria associated with diabetic nephropathy may be due in part to a reduction of albumin uptake at the tubule level. This decrease in albumin endocytosis and the rise of intratubular albumin concentration are proposed to be due to a reduction in megalin expression. Furthermore, NHE3 expression level is increased in models of diabetic nephropathy. Possible molecular mechanisms of activation in NHE3 transport function are presented. Lower level of megalin expression would result in a decrease in the amount of megalin in complex with NHE3 and the megalin-dependent NHE3 endocytosis. The increase in intratubular albumin concentration stimulates NHE3 expression. Furthermore, serum glucocorticoid-regulated kinase-1 (SGK1) expression is increased in animal models of diabetic nephropathy and cells treated with high glucose to mimic hyperglycemia. SGK is proposed as one of the signaling pathways activated to stimulate NHE3 transport function in diabetic nephropathy. This model requires further experimental validation. Dashed lines are used to indicate NHE3 transport activity or signaling pathways that remain to be fully defined.
transporting pool of NHE3 localized on the microvilli, while the pool associated with megalin and responsible for its endocytosis is localized in the intermicrovillar cleft (143). In diabetic nephropathy where megalin expression is decreased it is possible that more NHE3 is available to redistribute into the microvilli at the cell surface (Fig. 2B). Of note, this functional model matches nicely with that proposed for NHE3 regulation in hypertension. Additionally, SGK1 expression is increased in diabetic nephropathy and SGK1 stimulates NHE3 expression (Fig. 2B). Therefore, even more NHE3 will be delivered to the plasma membrane, and, in absence of megalin, even more NHE3 will migrate into the microvilli. A decrease in megalin expression would also be predicted to decrease albumin endocytosis and increase intratubular albumin concentration, which would further stimulate NHE3 expression and further aggravate the renal damage (Fig. 2B). This model of a potential physiologic role of the formation of NHE3-megalin complex in the proximal tubule of diabetic kidneys remains to be validated (143).

Upregulation of NHE3 Transport Activity in Heart Failure May Contribute to Edema Formation

Heart failure (HF) has become epidemic in the developed world and its prevalence is rapidly expanding in developing countries as well (87, 153). The incidence increases with age; it carries a worse prognosis than many cancers and lowers not only the length but also the quality of life of the patients (105). As such, HF has an overwhelming impact on global health and health care costs (127, 153). The rise in worldwide life expectancy and the fact that HF is a final common pathway for a host of cardiac disorders strongly suggest that the substantial healthcare expenditures and social impact associated with this syndrome will continue to escalate. Such aspects demand an effort from investigators to obtain a better understanding of the pathophysiology of this illness at molecular, cellular, organ, and clinical levels.

Edema is one of the most devastating clinical manifestations of HF. The most important cause of edema is salt and water retention by the kidneys (193, 194). Nevertheless, in edematous disorders, such as HF, renal dysfunction is not the primary cause of Na+ and water retention. In fact, the kidneys are functionally competent and do what is expected from them, i.e., respond as if the organism were facing a real hypovolemia.

The pathophysiology of salt and water retention in HF consists of inappropriate and persistent stimulation of the physiological systems that maintain extracellular volume homeostasis (26, 32). In response to myocardial injury or ventricular hemodynamic overload, various compensatory mechanisms are activated to maintain the pumping function of the heart and, consequently, cardiac output and tissue perfusion. Among these adaptive responses are neurohumoral activation of the sympathetic nervous system (SNS) and the renin-angiotensin-aldosterone system (RAAS), the Frank-Starling mechanism, and cardiac remodeling. On a short-term basis many of these mechanisms are adaptive, but they become maladaptive over the long term, causing progressive deterioration of the failing heart (41, 105).

Underfilling of the arterial system is a major hemodynamic abnormality in HF and is perceived as a volume deficit by the baroreceptors (26, 32). The activation of the arterial baroreceptors in the left ventricle, carotid sinus, and aortic arch transmit an afferent signal to decrease gasserian and glossopharyngeal inhibitory tone in the nervous central system, which in turn activates the SNS. The elevation of the renal adrenergic tone stimulates the RAAS, which promotes avid renal Na+ and water retention. Moreover, the activation of the SNS stimulates the paraventricular and supraoptic nuclei in the hypothalamus, leading to nonosmotic release of vasopressin, which by the activation of V2 receptors upregulates aquaporin-2 expression and apical membrane targeting, leading to an enhancement of renal water reabsorption in the distal nephron (26, 31, 32, 137, 150, 192).

Potential causes of renal salt retention include a decrease in GFR, an increase in tubular reabsorption of Na+, or both. Whereas hemodynamics and glomerular filtration have been extensively studied, much less is known about tubular handling of Na+ in HF.

Recent studies have been conducted to examine whether increased abundance of the main apical Na+ transport proteins plays a role in the pathogenesis of Na+ and water retention in HF. It has been reported that expression of the Na+-K+-2Cl- cotransporter (NKCC2) in the thick ascending limb is significantly increased in rats with HF at different stages of the disease (137, 151, 192, 205). Furthermore, studies from Lutken and colleagues (137) have shown that rats with HF display increased protein abundance of NHE3 as well as of the epithelial sodium channel (ENaC α-subunit in the renal cortex and outer medulla compared with sham rats.

Most recently, Inoue et al. (100), using stationary in situ microperfusion and pH-dependent Na+ uptake, showed that NHE3-mediated Na+/H+ exchange is higher in proximal tubules from rats with experimentally induced HF than from sham. These investigators have also demonstrated that the increase in NHE3 transport activity in HF goes along with enhanced NHE3-mRNA and total protein expression in the renal cortex. Moreover, rats with HF display increased NHE3 expression in the microvilli domain of the brush border which is paralleled by a reduction in the phosphorylation of NHE3 at the PKA consensus site serine 552, suggesting that NHE3 redistributes from subapical storage pools to the body of the renal microvilli in HF. Therefore, regulation of NHE3 in HF apparently occurs at transcriptional, translational, and post-translational levels and may contribute to the fluid retention characteristic of HF (Fig. 3).

Upregulated function and expression of NHE3 in HF may be driven by angiotensin II and/or norepinephrine (Fig. 3), since these neurohumoral factors are elevated in response to reduced cardiac output and both have been shown to increase NHE3 activity, at least in part, through increase of NHE3 protein expression (190, 206, 220). In fact, it has been demonstrated that elevated NHE3 and Na+ excretion are normalized in HF rats treated with angiotensin II receptor antagonists (137, 192). Whether norepinephrine alters renal sodium handling in HF via NHE3 remains to be determined.

Activation of RAAS and/or SNS may also decrease the endogenous levels of NHE3 phosphorylation. There is some evidence suggesting that binding of angiotensin II to the AT1 receptor decreases adenylyl cyclase activity, inhibits cAMP formation, and stimulates transcellular sodium transport in OK cells (200). Likewise, β-2-adrenoceptors associate with NHERF1, a cofactor that is necessary for PKA-mediated phos-
phosphorylation of NHE3 (230). Hall and colleagues (78) have demonstrated that ligand activation of β-2-adrenoceptors sequesters NHERF1 and relieves the baseline inhibition of NHE3 by PKA. It is noteworthy to mention that regulation of NHE3 via a cAMP pathway downstream of AT1 receptor activation is still merely speculative. Thus, further work needs to be done to establish the role of angiotensin II and/or norepinephrine in mediating the transcriptional, translational, and/or posttranslational regulation of proximal tubule NHE3 in experimental HF.

The interaction between the heart and the kidney, especially with respect to the control of the extracellular volume, arterial pressure, vascular tone, and tissue perfusion, is mediated not only by the integrated actions of both SNS and RAAS, but also by the peptidergic systems, including atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP). ANP and BNP are produced in the myocardial cells of the atrium and ventricle, respectively, and are secreted in response to distention of the cardiac chambers and/or arterial pressure elevation. These peptides play a central role in modulating extracellular volume homeostasis and blood pressure by counteracting the actions of SNS and RAAS (144, 169, 219). Plasma levels of ANP and BNP are increased in patients with HF and positively correlate with the degree of left ventricular dysfunction (25, 38, 156, 169, 204, 228). Indeed, BNP has been widely used as a biomarker for HF and its severity (43, 123). It is noteworthy that despite increases in the circulating levels of BNP and of other natriuretic peptides, renal Na⁺ retention, volume expansion, and edema persist in patients with HF (30, 102). Several mechanisms have been proposed to explain the hyporesponsiveness to natriuretic peptides in HF (26, 30), including 1) downregulation of natriuretic peptide receptors; 2) secretion of inactive natriuretic peptides; 3) increase of the proximal tubule Na⁺ reabsorption with resultant decrease of the Na⁺ delivery to the distal nephron where natriuretic peptide receptors are located; and 4) increased activity of peptidases that degrade and inactivate these natriuretic peptides and or decreased activity of peptidases that activate them. The recent published report by Inoue et al. (100) corroborates the third hypothesis.

Salt and water retention by the kidneys worsens long-term prognosis in patients with HF by accelerating progressive deterioration of the failing heart. Furthermore, most of the symptoms that impair quality of life in HF patients result in large measure from the patients’ inability to excrete salt and water. Clearly, further studies are needed to improve our understanding of the kidney’s adaptive and maladaptive responses to cardiac dysfunction, at multiple hierarchical levels.

**NHE3 Regulation in Acute Kidney Injury: Damage vs. Protection**

Acute kidney injury (AKI) is the most common syndrome in clinical nephrology (3, 92). Clinical outcomes of this syndrome have not improved over the past 50 years and have high morbidity and mortality despite advances in multiple fronts on overall clinical care (3, 92, 199, 210). Thus, AKI costs are very high, over $10 billion per year in the United States alone (34). Furthermore, AKI frequently develops to chronic kidney disease and associates with other severe complications, such as sepsis and multi-organ failure (e.g., HF) (94, 104, 108). Regrettably, therapeutic options to treat or prevent AKI, until recently, consisted of largely supportive measures (59, 210). Therefore, new therapeutic and preventive measures for AKI are under intense investigation. A thorough understanding of the pathophysiology of AKI is critical for further advances in the treatment of this disorder.

AKI is described as a rapid decline in kidney function (occurring over a period of minutes to days), resulting in the retention of metabolic waste products and often severely re-
duced urine output (oliguria). The leading causes of AKI are renal ischemia and reperfusion injury (IRI), sepsis, and nephrotoxin injury especially for surgical patients (94, 186). Among the principal cause of AKI, IRI is widely studied using very reproducible experimental approaches. Essential mechanisms of IRI include inflammation, endothelial and renal cell necrosis and apoptosis. Other leading causes of AKI are nephrotoxic agents such as radiocontrast dyes, antibiotics, chemotherapeutics, and heavy metals (94, 154). These nephrotoxins are concentrated by reabsorption in the kidney tubular lumen and have direct toxic effects on the tubules. AKI also occurs in patients with sepsis (94). Frequently, AKI manifests with damages of other organs such as the heart (154, 159); indeed, extra-renal complications are the principal causes of mortality in AKI.

In IRI the decrease in urine output is associated with reduced GFR, but it may follow a paradoxical oliguric phase with increased salt excretion secondary to acute tubular necrosis (20, 29, 185). The natriuresis occurring as a result of IRI would involve a reduction in renal Na⁺ reabsorption along the nephron. Indeed, the abundance of several major kidney Na⁺ transporters, including NHE3, is severely reduced after IRI in rats (121, 215, 216). Similarly, lipopolysaccharides-induced septic AKI increases fractional Na⁺ excretion and strongly reduced NHE3 protein expression (183). In addition, it was shown that NHE3 surface expression, total protein and transcript abundance are all depressed (51) and return to control levels after about 7–10 days congruent with the recovery of GFR (98). The temporal relationships indicate distinct mechanisms operating in parallel: decreased NHE3 in the apical membrane precedes the decrease in total protein, which precedes the decrease in NHE3 transcript.

The proximal tubule NHE3, responsible for reabsorbing the bulk of the filtered load, is particularly susceptible to ischemic insult (4). Therefore, severe suppression of NHE3 in IRI may constitute a pathophysiological event that can potentially worsen the clinical syndrome. Indeed, it could represent, at least in part, the molecular basis for the natriuresis, acidosis and volume depletion observed after IRI (215, 216), though salt wasting and disordered acidification are not commonly encountered in AKI (8).

Interestingly, the reduction in NHE3 expression is not necessarily associated with renal tubular necrosis or loss of apical membrane structure, but is globally distributed (51). These observations may be key in the understanding the pathophysiology of AKI, especially considering that tubular necrosis of AKI is extremely patchy in humans (130). In support of these findings, pretreatment with the NHE inhibitor EIPA [5-(N-ethyl-N-isopropyl) amiloride] attenuates Na⁺ fractional excretion and renal endothelin-1 released in response of IRI (222). Pharmacologic inhibition of NHE3 by S3226 blunts the rise in serum creatinine after IRI and improves AKI (96). Systemic administration of another specific inhibitor of NHE3 also improves tubular function in AKI and provides protection from acute rejection in renal transplant in rats (172).

Overall, these findings support the alternative but not necessarily exclusive hypothesis that downregulation of NHE3 is not a consequence of kidney damage but rather an adaptive defense mechanism. This notion is sustained by the following two viewpoints. 1) Conservation of energy by the kidney (18): Active Na⁺ transport constitutes the bulk of adenosine triphosphate (ATP) consumption (110), especially in the largely aerobic proximal tubule (9, 177) (Fig. 4A). A rapid reduction in Na⁺ transport mediated by depressing apical Na⁺ entry would rapidly achieve that purpose (Fig. 4B). Furthermore, reduction in apical Na⁺ entry may be cytoprotective to the cell as low cell Na⁺ accelerates Ca²⁺ efflux and ameliorates Ca²⁺-mediated cell injury (22). 2) Conservation of isotonic extracellular fluid (202): It is critical that GFR is reduced to match the decreased transport capacity to prevent massive volume loss. The reduction in GFR in acute tubular necrosis can be partially accounted for by increased solute delivery to the macula densa and tubuloglomerular feedback (TGF) (17). By analogy, in NHE3-deficient animals, loss of NHE3 results in severe reduction of GFR secondary to activation of TGF, a mechanism to prevent excess excretion and circulatory collapse (136). Hence, downregulation of NHE3 may be a fundamental mechanism of kidney protection (Fig. 4, A and B) rather than an undesirable consequence of the tubular response to IRI. Understanding the molecular mechanisms by which IRI regulate NHE3 expression is critical to aid understanding the renal response to AKI.

What are the molecular mechanisms that couple AKI due to IRI to reduced NHE3 (121, 215, 216)? Urinary NHE3 protein abundance was found to be increased in patients with acute tubular necrosis compared with normal subjects (56). Similarly, urinary NHE3 protein abundance was found to be increased early in septic AKI (10). These are important findings that may suggest that NHE3 reduction in IRI is due, at least in part, to its shedding in urinary exosomes. Additionally, these findings support the investigation of the use of NHE3 antigen level as a urinary biomarker in AKI. Currently, it is not clear whether NHE3 antigen in urinary exosomes actually reflects renal levels based on constitutive shedding or discharge through necrosis in the setting of tubular necrosis.

Furthermore, an abrupt decrease in NHE3 expression was measured in OK cells never exposed to hypoxia but treated with homogenates from ischemic animals (51). This decrease in total NHE3 protein was reversed by treatment of OK cells with the proteasomal pathway inhibitor lactacystin but not with the lysosomal pathway inhibitor leupeptin, leading to the conclusion that NHE3 degradation was mediated via the ubiquitin-proteasome proteolytic system (51). The two major proteolytic systems described in eukaryotic cells are the proteasomal and lysosomal pathways (125, 155), and ubiquitin plays a major role in protein degradation as it serves as a tag for recognition of proteins by the proteolytic pathway (88–90) (Fig. 4B). This reconstitution experiment suggests the possibility that loss of NHE3 protein after IRI is due, at least in this cell model, to production of factor(s), retained in the homogenates from ischemic animals, that triggers NHE3 tagging and degradation.

In OK cells treated with homogenates from ischemic animals, NHE3 protein and transcript expression as well as NHE3 at the cell surface are likely affected by distinct mechanisms that correlate with the degree of kidney damage. Indeed, ischemic extracts obtained for animals with mild ischemia added to OK cells reduced only cell surface NHE3. On the other hand, extracts from more severe renal damage decreased total as well as surface NHE3 protein and NHE3 transcripts. These findings support the view that diffusible factor(s), released or activated in or near the tubular epithelium in IRI, may play a significant role in the reduction of NHE3 expression.
The identity of these diffusible factor(s) has not been defined thus far but evidence suggests that the factors are heat-labile and likely composed of lipid and protein or protein in lipid-enriched domains, but not regulatory RNA components (51). Modifications in lipid content and microRNA expression (an important regulator of gene expression) (15) have been associated with kidney dysfunction in AKI (19, 70).

What are the candidate extracellular and/or intracellular diffusible factor(s) that could regulate NHE3 protein expression in IRI? An increasing body of evidence indicates the inflammatory response as a major player in IRI (112). The inflammatory cascades started by endothelial dysfunction can be increased by the ischemia in the proximal tubule; these include release of proinflammatory (e.g., TNF-α, IL-6, IL-1β, and TGF-β) and chemotactic cytokines (e.g., monocyte chemotactic protein-1, IL-8, RANTES) (23). TNF-α and IL-1β, especially in combination, extensively downregulate the expression of several Na+ transporters, including NHE3 (183). Of interest, low dose of lipopolysaccharides, which causes a release of proinflammatory cytokines, inhibits NHE3 protein expression (183). In contrast, α-MSH, a potent antiinflammatory agent that inhibits the migration of neutrophil and the production of neutrophil chemokines (118), reverses the reduced abundance of Na+ transporters (e.g., NHE3) and the increase in fractional Na+ excretion induced by IRI (71). Beside release of extracellular factors, intracellular mediators also allow cells to respond and adapt to low oxygen tension. Hypoxia-inducible factors facilitate both oxygen delivery and adaptation to oxygen deprivation by regulating several cellular mechanisms such as intracellular pH via induction of NHE1 expression (77, 187).

In summary, renal IRI reduces both NHE3 surface and total protein abundance as well as NHE3 transcript expression. Recruitment of each of these mechanisms may occur via different pathways and may vary with degree of kidney damage. NHE3 shedding and NHE3 degradation are proposed as mechanisms of regulation of NHE3 transport function in IRI (Fig. 4B). Furthermore, the observation that reduced NHE3 protein is globally distributed in the kidney supports the hypothesis that downregulation of NHE3 at diverse levels may initiate a rapid mechanism to conserve energy and protect the cell; but this and the signaling pathways activated during IRI to inhibit NHE3 have not been proved thus far.

Pivotal Role of NHE3 in Acute and Chronic Diarrhea

According to the World Health Organization (WHO), diarrhea kills 2.2 million people every year, mostly children in developing countries (1). This mortality rate has declined from four decades ago, when diarrhea was estimated to have caused at least five million deaths per year in children under the age of five alone. However, every year, in every developing country,
Diarrhea continues to be one of the leading causes of childhood deaths (139, 179). Furthermore, in contrast to the decline in rates of mortality, there has been little if any reduction in the morbidity associated with diarrhea.

The implementation of oral rehydration therapy (ORT) to control the mortality rate associated with diarrhea is among the greatest public health accomplishments of the 20th century (149). During the 1940s, Darrow helped pave the way for physiological research by recognizing that “effective replacement of water and electrolyte in patients with clinical diarrhea should be based on exact knowledge of changes in composition of body fluids” (49). Physiological studies in the 1950s defined the coupled intestinal absorption of Na\(^+/\)H\(^+\) and glucose, two major constituents of ORT, laying the foundation for the clinical use of ORT (46, 60, 173). In 1968, after several years of intensive research in universities and in cholera fields, the first cholera patients were successfully treated with an ORT alone (179). The entwined stories of physiologists and clinicians and the fight against cholera that culminated in the development of ORT illustrate an early translational research success story (74, 179).

Diarrhea is a symptom of the response of the gastrointestinal tract to a wide range of perturbations, particularly enteric infections (141). It results from either a decrease in intestinal absorption or an increase in intestinal secretion which in turn leads to a net increase of fluid in the stool. As a consequence of excessive loss of water and electrolytes in the stool, affected individuals can develop severe dehydration and hypokalemia (84, 141). Consequences of diarrhea in children can include malnutrition, diminished growth, and cognitive impairment (74, 163).

Diarrhea is clinically classified based on duration and etiology. Most acute diarrheas last less than four weeks and are generally due to intestinal infection. Chronic diarrhea persisting for periods longer than a month are clinically divided into malabsorption syndromes, inflammatory bowel diseases such as Crohn’s disease or ulcerative colitis, irritable bowel disease, and congenital diarrhea (141).

Neutral NaCl absorption occurs along the entire length of the intestine. In most diarrheas, neutral NaCl in the Na\(^+/\)H\(^+\) absorptive cell is inhibited and electrogenic chloride secretion from the crypt cells is stimulated. Not surprisingly, NHE3 null mice suffer from mild chronic diarrhea (184). In fact, regardless of etiology, diarrhea is almost always at least partially caused by inhibition of NHE3 activity.

The leading causes of diarrhea include intestinal infections with *Vibrio cholerae*, *Escherichia coli*, and rotavirus. The cholera toxin binds to the membrane-associated ganglioside GM1 located at the apical membrane of the small intestine, is endocytosed, and traffics via endosomes to the Golgi and subsequently to the endoplasmic reticulum (ER) where the A1 chain of the CT is unfolded and subsequently released from the ER into the cytosol. The A1 chain activates the \(\alpha\)-subunit of a G protein (Gs) which in turn increases adenylyl cyclase activity and leads to elevation of the intracellular concentration of cyclic adenosine monophosphate (cAMP). The resulting increase in cAMP activates PKA that increases anion secretion by stimulating the activity of the chloride channel (CFTR) and decreases fluid absorption by inhibiting the activity of NHE3. Severe diarrhea induced by CT is therefore mediated by decreased absorption and increased secretion of electrolytes and solutes in the intestine. Oral rehydration solutions contain glucose, Na\(^+\), Cl\(^-\), and HCO\(_3\)\(^-\) and are extremely efficient in enhancing fluid and electrolyte absorption in severe diarrhea. The scientific basis of oral rehydration therapy consists in the fact that the function of the Na\(^+/\)H\(^+\) -coupled glucose transporter, SGLT1, is not altered by the CT. The efflux of glucose across the basolateral membrane occurs by facilitated diffusion via the glucose transporter GLUT2. Intestinal fluid absorption follows the movement of the nutrients. Stimulation of NHE3 by SGLT1-mediated glucose absorption (132) may also contribute to Na\(^+\) absorption during oral rehydration therapy, but its quantitative contribution has not been established.

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**Fig. 5.** Role of NHE3 as a downstream target of cholera toxin (CT)-mediated diarrhea and as possible contributor to intestinal sodium absorption during oral rehydration therapy. The CT produced by the *Vibrio cholerae* binds the membrane-associated ganglioside GM1 located at the apical membrane of the small intestine, is endocytosed, and traffics via endosomes to the Golgi and subsequently to the endoplasmic reticulum (ER) where the A1 chain of the CT is unfolded and subsequently released from the ER into the cytosol. The A1 chain activates the \(\alpha\)-subunit of a G protein (Gs) which in turn increases adenylyl cyclase activity and leads to elevation of the intracellular concentration of cyclic adenosine monophosphate (cAMP). The resulting increase in cAMP activates PKA that increases anion secretion by stimulating the activity of the chloride channel (CFTR) and decreases fluid absorption by inhibiting the activity of NHE3. Severe diarrhea induced by CT is therefore mediated by decreased absorption and increased secretion of electrolytes and solutes in the intestine. Oral rehydration solutions contain glucose, Na\(^+\), Cl\(^-\), and HCO\(_3\)\(^-\) and are extremely efficient in enhancing fluid and electrolyte absorption in severe diarrhea. The scientific basis of oral rehydration therapy consists in the fact that the function of the Na\(^+/\)H\(^+\) -coupled glucose transporter, SGLT1, is not altered by the CT. The efflux of glucose across the basolateral membrane occurs by facilitated diffusion via the glucose transporter GLUT2. Intestinal fluid absorption follows the movement of the nutrients. Stimulation of NHE3 by SGLT1-mediated glucose absorption (132) may also contribute to Na\(^+\) absorption during oral rehydration therapy, but its quantitative contribution has not been established.
Escherichia coli produces two distinct enterotoxins that induce fluid and electrolyte secretion through two distinct receptors and second-messengers systems. The heat-labile E. coli toxin binds to an apical membrane receptor, becomes internalized, and, like cholera toxin, activates basolateral adenyl cyclase. The heat-stable toxin of E. coli binds to and activates guanylyl cyclase on the apical membrane. The resultant increase in cyclic guanosine monophosphate (cGMP) activates PKG.

The rotavirus nonstructural protein (NSP4) functions as a viral enterotoxin and is essential for virus pathogenesis (188). After retrovirus invasion, the NSP4 toxin is translated, and secreted from the epithelial cell into the lumen where NSP4 diffuses into the crypts and binds to a specific receptor which stimulates phospholipase C (PLC). PLC generation leads to the production of inositol 1,4,5-triphosphate (IP3) and the release of calcium from intracellular stores (52).

All three toxin-stimulated second-messenger systems—cAMP, cGMP, and calcium—inhibit NHE3-mediated NaCl electroneutral absorption; a number of reports exist on the inhibitory effect of toxins that cause diarrhea on NHE3 function (33, 73, 81, 85, 93, 132, 196). However, no information is available about the possible role of NSP4 in regulating NHE3 activity.

The mechanisms by which choler toxin inhibits NHE3 activity in intestinal cells has been reported by Subramanya et al. (196). Specifically, they discovered that 5-h exposure of rat ileal loops to choler toxin reduced NHE3 protein expression by ~60% but did not significantly alter NHE3-mRNA levels (196). This study also revealed that 2-h incubation of rat ileal loops with the bacteria-derived short chain fatty acids (SCFAs) butyrate, following choler toxin, decreased cAMP levels and completely restored NHE3 activity and protein expression.

SCFAs such as acetate, propionate, and butyrate are produced by fermentation of unabsorbed carbohydrates (e.g., amylase-resistant starch) by colonic bacteria. In the colon, SCFAs stimulate NHE3-mediated NaCl and fluid absorption (148). It has been previously demonstrated that SCFA-dependent Na+ absorption is not affected by cAMP (166) and that SCFAs are capable of reversing choler-induced decreased fluid absorption in the large intestines (165). These observations led Binder and colleagues (167) to propose that addition of amylase-resistant starch to the ORT (to stimulate SCFA production) would result in reduced stool volume and a shorter duration of diarrhea in choler-infected patients. The direct test of this hypothesis demonstrated that addition of resistant starch to the ORT not only improved rehydration but also decreased the duration of diarrhea and loss of fluid in the stool of patients with cholera (167).

In a recent study, Lin et al. (132) found that a nonmetabolizable substrate of the Na+/glucose cotransporter-1 (SLGT1), α-methyl-D-glucose (α-MD-G), stimulated jejunal NHE3 activity in a model of acute choler toxin-induced watery diarrhea, corroborating the findings that agents that stimulate NHE3 function may reverse the cholera toxin-induced cAMP-mediated inhibition of NaCl absorption. In Caco-2 cells, activation of NHE3 activity by α-MD-G was demonstrated to be due to increased NHE3 surface expression (132). Subcellular redistribution of NHE3 from subapical storage pools to the microvilli in response to α-MD-G involves dissociation of the NHE3 and NHERF2 complex which retains NHE3 in a subapical domain. The response to α-MD-G is dependent on the Akt signaling pathway (132).

In ileal and colonic pinch biopsies from patients with inflammatory bowel diseases (IBD), the protein abundance of NHE3 as well as of NHERF1 and NHERF2 was reduced compared with normal subjects (197). Similar findings were encountered in the colon of DSS (dextran sodium sulfate) and TNBS (2,4,6-trinitro benzene sulfonic acid)-induced murine IBD models (197). Additionally, in interleukin-2 deficient mice, a model of ulcerative colitis, reduced NHE3 activity was paralleled by decreases in both NHE3 protein and mRNA levels, explaining, at least in part, the remarkably low levels of NaCl absorption in these mice (13). Moreover, the two major proinflammatory cytokines associated with intestinal inflammation, TNF-α and IFN-γ, repress the NHE3 promoter activity in human intestinal epithelial cells (7). On the other hand, despite significant reduction of NHE3 activity, neither changes in NHE3 protein or mRNA expression were found in colonic biopsies from ulcerative colitis patients compared with controls (226). In addition, jejunal NHE3 activity is reduced by ~80% in an experimental mice model of TNF-α-mediated diarrhea and this inhibition is largely mediated by a mechanism that involves PKC-mediated NHE3-internalization (37). In any event, all existing evidence points to a pivotal role of NHE3 in diarrhea associated with inflammatory bowel diseases.

Congenital Na+ diarrhea (CSD) is an extremely uncommon inherited secretory diarrhea characterized by dehydration, metabolic acidosis, and hyponatremia due to remarkable loss of Na+ and bicarbonate in the stools. Booth et al. (24) were the first to demonstrate that CSD is caused by impaired jejunal brush border Na+/H+ exchange. These findings were based on measurements of pH-dependent Na uptake into brush border membrane vesicles prepared from jejunal biopsies from control and affected subjects in which NHE activity in patients was reduced to less than 10% of that in controls (24, 107). A molecular explanation for CSD remains to be determined, since gene-inactivating mutations of NHE3 or of other Na+/H+ exchangers cannot account for this disorder (14, 147). Thus, impaired apical Na+/H+ exchange in the intestine of CSD patients might be due to a derangement in a NHE3 regulatory protein.

Persistent questions remain about etiology and treatment of diarrheal disorders, and these queries keep furthering gastrointestinal physiology. Nonetheless, strides have been made in the understanding of the role of NHE3 and its regulatory partners as well as of other multiprotein complexes of transporters, crucial to the processes of intestinal absorption and secretion. These strides have provided insights needed to develop new therapies and resulted in significant improvements in the management of diarrhea, especially in children. Therefore, apart from the socioeconomic constraints that pre-
clude reduction of intestinal infections in developing countries, the cycle of clinical observations, physiological research, and improvements of clinical outcomes has been persistent and successful.

Function of NHE3 in Pathophysiological States: Conclusion and Prospective

It is clear that restoring the ion balance in clinical conditions where it is compromised is essential to blunt or reverse progressive organ deterioration and damage. Therefore, studying the molecular mechanisms activated to regulate NHE3 and their deviations as a result of pathophysiological conditions is indispensable for deciphering the organ response to the specific disorders and to establish new therapies. Table 1 summarizes the mechanisms of regulation of NHE3 in hypertension, diabetic nephropathy, HF, AKI, and diarrhea, which were analyzed in this review. It is evident that NHE3 is regulated at multiple levels, starting from changes in transport activity and trafficking along the microvilli. However, levels of surface and total NHE3 are also regulated together with mRNA expression (Table 1). These observations indicate that each and every potential mechanism to regulate NHE3 transport function is recruited to respond to and/or as a result of the specific pathophysiological state. This prompts the question as to what is orchestrating the recruitment of one mechanism vs. another one or their dynamic convergence in regulating NHE3. For instance, in hypertension it is mainly NHE3 activity, its phosphorylation status, and its trafficking that are modulated while in HF, diabetic nephropathy and AKI NHE3 protein and mRNA levels are affected (Table 1). Is it the duration of the stimuli or the activation of the specific upstream signaling pathway that determines the recruitment of one mechanism vs. the other one? Is the regulation of NHE3 transport function in one pathophysiological state correlated to damage while in the other to protection? Or could it be that the prolonged and continued activation of molecular machineries normally triggered only by an acute response may be caused by or even cause the disease? Despite the remarkable amount of findings that have accumulated in the past few decades, these fundamental questions are far from being answered. It is also far from being understood the impact of NHE3 regulation in the different clinical conditions. As discussed, edema, one of the most devastating clinical manifestations of HF, is provoked by salt and water retention by the kidneys (193, 194). Therefore, activation of NHE3 transport function in HF (Table 1) could contribute to exacerbation of edema and the use of specific NHE3 inhibitors may attenuate the salt and water retention and improve the action of natriuretic peptides in HF. It is unlikely that pharmacological blockade of NHE3 will alone completely prevent volume overload in HF because edema formation may be affected by hemodynamic mechanisms or activation of other sodium transporters. Nevertheless, the combination of NHE3-specific inhibitor with inotropic agent could improve the function of the heart in HF, though this remains to be established. Furthermore, NHE3 is strongly reduced in AKI due to IRI, and AKI is a common complication in patients with cardiovascular disease such as HF (94). The effect that NHE3 inhibitor may have on HF with AKI complication also remains to be investigated.

In summary, NHE3 research in human diseases has yet to reach its full potential. Vast areas of investigation remain to be explored, including explaining the transport machineries in molecular details, establishing the relevance of details to clinical disorders, and using them to design specific therapeutic interventions.

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AUTHOR CONTRIBUTIONS

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C1584 FUNCTION OF THE Na\(^{+}/\)H\(^{+}\) EXCHANGER-3 IN CLINICAL DISORDERS


