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## Regulation of Na<sup>+</sup>/H<sup>+</sup> Exchanger Isoform 3 by Protein Kinase A in the Renal Proximal Tubule

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### 1. Introduction

One of the major functions of the kidneys is to maintain the volume and composition of the body fluids constant despite wide variation in the daily intake of water and solutes. To accomplish this task, the activities of a number of transport proteins along the nephron are tightly regulated.

The nephron is the functional unit of the kidneys. Each human kidney contains approximately 1.2 millions of nephrons. At the beginning of each nephron, in the glomerulus, the blood is filtered: cells and most proteins are retained, whereas water and small solutes pass from the glomerular capillaries to the Bowman's capsule. As the glomerular filtrate leaves Bowman's capsule and enters the renal tubule, it flows sequentially through the proximal tubule, the loop of Henle, the distal tubule, and the collecting duct. Along this course, greater part of the glomerular filtrate is transported across and between the tubule cells and reenters the blood (reabsorption), whereas some is secreted from the blood into the luminal fluid (secretion). The formation of urine involves the sum of these three major processes: ultrafiltration of plasma by the glomerulus, reabsorption of water and solutes from the ultrafiltrate, and secretion of solutes into the tubular fluid. Although 180 liters of plasma is filtered by the human glomeruli each day, less than 1% of water, sodium chloride and variable amounts of other solutes are excreted in the urine. By the processes of reabsorption and secretion the renal tubule modulates the volume and composition of the urine. Consequently, the tubules precisely control the volume, composition, and pH of the body fluids.

The renal proximal tubule is responsible for reabsorption of the majority of the filtered sodium, bicarbonate, chloride and water. Na<sup>+</sup>/H<sup>+</sup> exchange is the predominant mechanism for absorption of Na<sup>+</sup> and secretion of H<sup>+</sup> across the apical membrane of proximal tubule cells (Alpern, 1990). Apical membrane Na<sup>+</sup>/H<sup>+</sup> exchange also has a major role in mediating chloride reabsorption in the proximal tubule through its combined activity with a Cl<sup>-</sup>/base exchanger and by creating an increase in luminal chloride concentration that favors the diffusion of the anion from the tubular lumen to the blood (Warnock and Yee, 1981; Aronson and Giebisch, 1997). The sodium/proton exchanger isoform 3 (NHE3) represents

the major topic of this chapter; therefore the properties of the NHE family will be briefly discussed below.

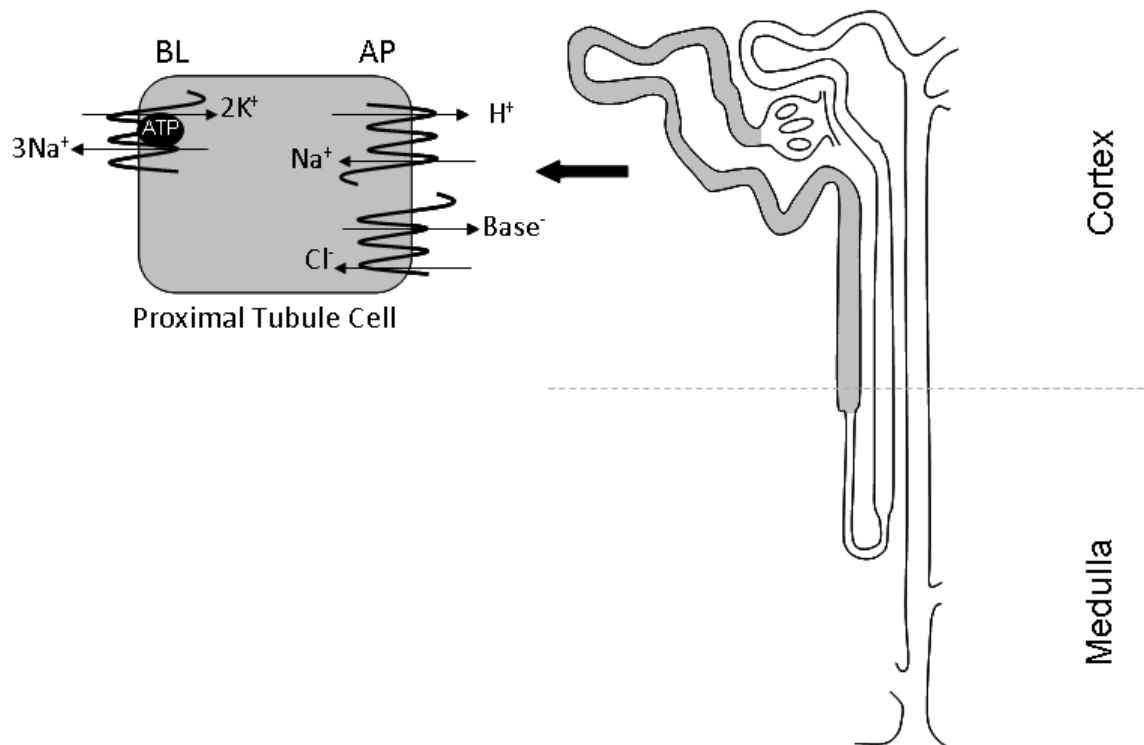


Fig. 1. Schematic illustration of the nephron depicting the most important transport mechanisms involved with NaCl reabsorption in proximal tubule. The inset shows Na<sup>+</sup>/K<sup>+</sup>-ATPase, Na<sup>+</sup>/H<sup>+</sup> exchanger and Cl<sup>-</sup>/Base<sup>-</sup> exchanger localization in proximal tubule. BL = basolateral; AP = apical.

The mammalian Na<sup>+</sup>/H<sup>+</sup> exchanger (NHE) gene family (SLC9) consists of secondary active transporters that mediate the electroneutral exchange of intracellular protons for extracellular sodium (Aronson, 1985). The transport activity of this protein is crucial to regulation of intracellular pH and cellular volume. In polarized epithelia, Na<sup>+</sup>/H<sup>+</sup> exchangers are also involved in transepithelial NaHCO<sub>3</sub> and NaCl transport.

All members of the NHE family share a common structural feature. They consist of two major portions, an N-terminal transmembrane domain and a large cytoplasmic C-terminal domain. The N-terminal portion of all known isoforms is predicted to span the plasma membrane twelve times. This domain is responsible for the Na<sup>+</sup>/H<sup>+</sup> exchange transport function (Pouyssegur, 1994). The C-terminal portion is mainly hydrophilic and it is the portion through which the activity of the exchanger is regulated.

Thus far, five sodium proton exchangers (NHE1, NHE2, NHE3, NHE4 and NHE8) have been identified in plasma membrane of renal tubular cells (Biemesderfer et al., 1992; Biemesderfer et al., 1993a; Amemiya et al., 1995; Chambrey et al., 1997; Chambrey et al., 1998). Of these, NHE3, the most abundant NHE isoform in renal tissue, is confined to the apical membrane of proximal tubule and thin and thick ascending limb. Several lines of evidence strongly support the conclusion that NHE3 is the principal NHE isoform responsible for apical membrane Na<sup>+</sup>/H<sup>+</sup> exchange in the proximal tubule. First, studies

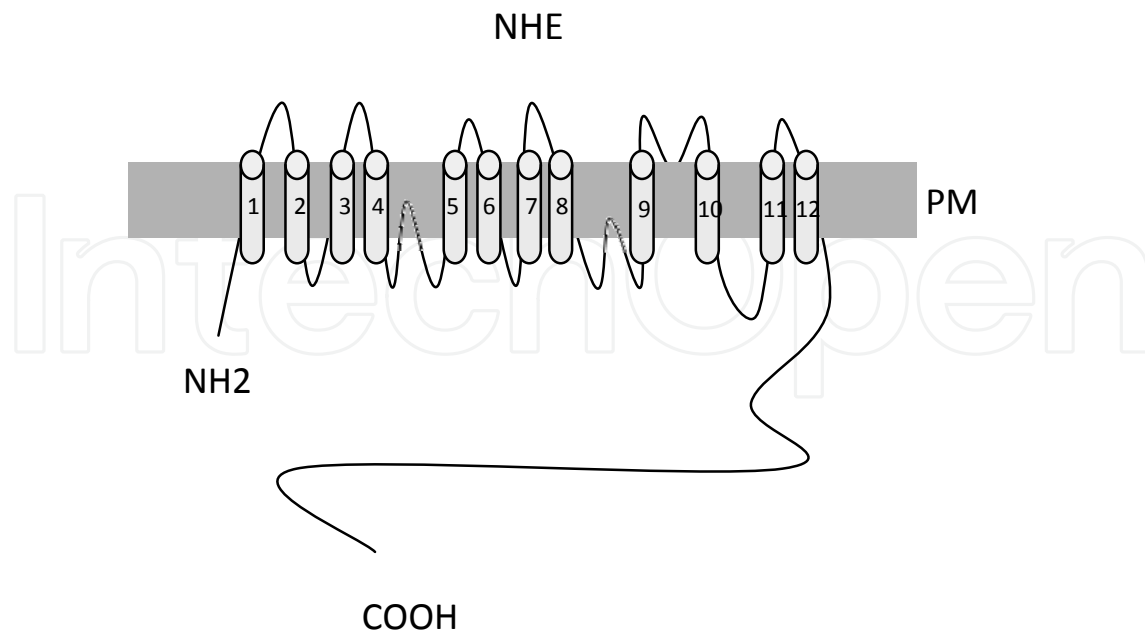


Fig. 2. Secondary structure of sodium/proton exchangers (NHE). All NHE isoforms have a membrane topology of 10-12 transmembrane segments. The carboxy-terminal region at the cytoplasmic site of NHE3 has consensus phosphorylation sites for PKA.

using isoform-specific antibodies have demonstrated that NHE3 is expressed on the brush border membrane of proximal tubule cells (Biemesderfer et al., 1993b; Biemesderfer et al., 1997). Second, the pattern of sensitivity to different inhibitors of Na<sup>+</sup>/H<sup>+</sup> exchange in renal brush border vesicles and of bicarbonate absorption in microperfused proximal tubules is most consistent with the properties of NHE3 among the known NHE isoforms (Vallon et al., 2000). Third, micropuncture analysis on NHE3 knockout mice revealed a remarkable reduction of fluid and bicarbonate reabsorption in renal proximal tubule further supporting the concept that NHE3 is the isoform that accounts for most Na<sup>+</sup>/H<sup>+</sup> exchange in this nephron segment (Lorenz et al., 1999; Wang et al., 1999). Indeed, mice lacking NHE3 display hypotension and had a mild hyperchloremic metabolic acidosis (Schultheis et al., 1998). Despite the reduced salt reabsorptive capacity in the renal proximal tubule, the NHE3 deficient mice grows well when fed a normal sodium diet, mostly due to reduced glomerular filtration rate and increased sodium and bicarbonate reabsorption in the distal nephron. However, if these animals are subjected to dietary salt restriction the adaptative mechanisms are not sufficient to fully compensate for the large defect on proximal reabsorption and they may die from hypovolemic shock (Ledoussal et al., 2001).

Given the important role of NHE3 in mediating NaHCO<sub>3</sub> and NaCl reabsorption in the proximal tubule, this transporter is subject to acute and chronic regulation in response to a variety of conditions and humoral factors affecting acid-base or salt balance. In this chapter we will focus on the regulation of NHE3 by protein kinase A and the implications of this regulatory mechanism on renal function under physiological and pathophysiological conditions.

## 2. Regulation of NHE3 activity by hormones that activate cAMP-dependent protein kinase A (PKA) in renal proximal tubule

The signal transduction cascade mediating the acute effect of NHE3 agonists and antagonists involves multiple pathways. One of the best studied regulatory mechanisms affecting NHE3 activity is the inhibition resulting from protein kinase A (PKA) activation. Hormones activating cAMP-dependent PKA have been shown to reduce sodium and bicarbonate reabsorption in renal proximal tubule by inhibiting NHE3 transport activity. Table 1 presents a summary of hormones and molecular mechanisms associated with inhibition of NHE3 by PKA. These hormones act via G-protein coupled receptors (GPCR) expressed in the apical membrane of the renal proximal tubule (Felder et al., 1984; Muff et al., 1992; Marks et al., 2003; Schlatter et al., 2007; Crajoinas et al., 2011). GPCR signal transduction occurs through coupling to heterotrimeric G proteins on the intracellular side of the membrane. Heterotrimeric G proteins contain three subunits referred as  $G\alpha$ ,  $G\beta$ , and  $G\gamma$ . Upon ligand binding, the GPCR undergoes a conformational change that promotes the exchange of bound guanosine diphosphate (GDP) from the  $G\alpha$  subunit for guanosine triphosphate (GTP). The G protein  $G\alpha$  subunit bound to GTP can then dissociate from the  $G\beta\gamma$  dimer and initiate the intracellular signaling cascade that leads to cAMP dependent PKA activation that further elicits NHE3 inhibition (Fig. 3).

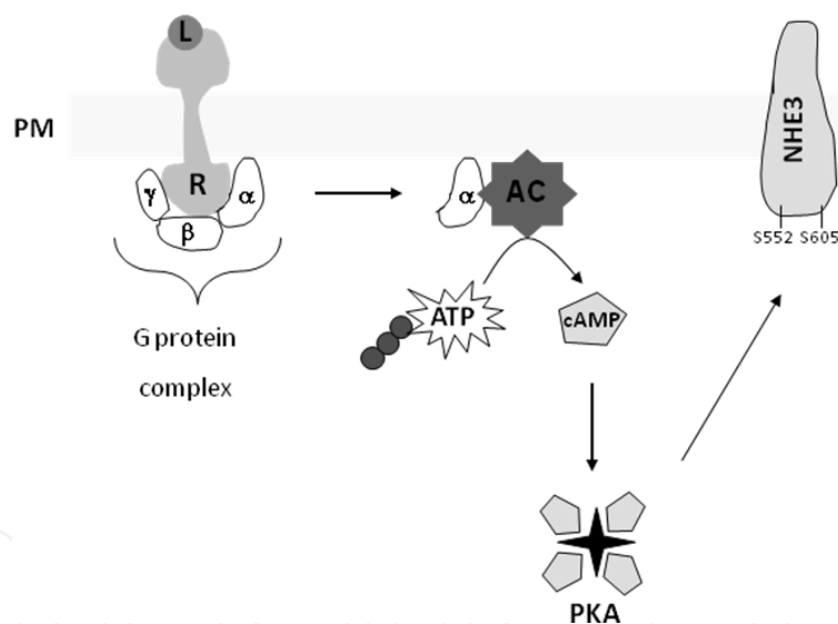


Fig. 3. Downstream effect of a receptor coupled to adenylyl cyclase. The ligand (L) binds to its receptor coupled to protein G ( $\alpha_s$ ) and adenylyl cyclase (AC) is activated. The activated enzyme converts ATP to cAMP, which activates protein kinase A (PKA) which, in turn, is ready to phosphorylate NHE3 at the specific PKA consensus sites.

### 2.1 Parathyroid hormone

The kidney is a principal target organ for the action of the parathyroid hormone (PTH). PTH is primarily involved in modulation of serum calcium and phosphate homeostasis but also acts on the proximal tubule, the thick ascending limb, and the distal convoluted tubule to alter urinary electrolyte and fluid excretion. The inhibitory effect of PTH on renal proximal

Hormone/Condition	Associated Mechanism	References
Parathyroid hormone	<p>↓ of NHE3 affinity for protons due to phosphorylation of the exchanger</p> <p>↓ of NHE3 maximum velocity due to a decrement of the number of NHE3 molecules at the plasma membrane</p> <p>↓ of NHE3 promoter activity, NHE3 mRNA and protein abundance</p>	(Fan et al., 1999; Collazo et al., 2000; Zhang et al., 1999; Girardi et al., 2000; Bezerra et al., 2008)
Dopamine	<p>↓ NHE3-mediated Na<sup>+</sup>/H<sup>+</sup> exchange due to increased endocytosis caused by increased PKA-mediated NHE3 phosphorylation</p>	(Gomes & Soares-da-Silva, 2004; Hu et al., 2001; Bacic et al., 2003)
Glucagon-like peptide 1	↓ NHE3 activity due to increased NHE3 phosphorylation	(Crajoinas et al., 2011; Carraro-Lacroix et al., 2009)
Glucagon	<p>Acutely - ↓ NHE via a PKA-dependent pathway</p> <p>Chronically - ↑ NHE3 mRNA and protein expression at the plasma membrane</p>	(Amemiya et al., 2002)
Guanylins	↓ NHE3-mediated Na <sup>+</sup> /H <sup>+</sup> due to increased levels of NHE3 PKA-dependent phosphorylation and reduction of the exchanger at the plasma membrane	(Amorim et al., 2006; Lessa LM, Girardi AC, and Malnic G, unpublished observations)
High Salt Diet	↓ NHE3-mediated Na <sup>+</sup> /H <sup>+</sup> due to higher NHE3 phosphorylation on serine 552, redistribution from microvilli to the intermicrovillar region together with its regulatory partner dipeptidyl peptidase IV	(Yang et al., 2008)
Hypertension	↓ NHE3-mediated Na <sup>+</sup> /H <sup>+</sup> due to higher NHE3 phosphorylation on serine 552, redistribution of the transporter to the intermicrovillar region	(Magyar et al., 2000; Panico et al., 2009; Crajoinas et al., 2010)
Heart Failure	↑ NHE3-mediated sodium reabsorption due to increased renal cortical NHE3 mRNA and protein levels and lower levels of NHE3 phosphorylation at serine 552	(Lutken et al., 2009; Inoue et al., 2012)

Table 1. Major factors and hormones that inhibit NHE3 activity via cAMP-dependent PKA activation.

tubule NHE3 activity has been consistently reported by several laboratories on both *in vivo* (Bank and Aynedjian, 1976; Girardi et al., 2000) and *in vitro* studies (Pollock et al., 1986; Mrkic et al., 1992; Mrkic et al., 1993). Experiments performed *in vitro* and *ex vivo* provide evidence that the acute inhibition of NHE3 by PTH is mediated by molecular mechanisms that include reduction of the transporter's apparent affinity for protons in consequence of the direct phosphorylation of the exchanger followed by reduction of its maximum velocity due to a decrement of the number of NHE3 molecules expressed at the plasma membrane (Fan et al., 1999; Collazo et al., 2000). Consistent with a decrease of NHE3 surface expression in response to PTH, studies carried out by the McDonough laboratory have shown that reduction of NHE3 activity in response to acute treatment with this hormone is a consequence of NHE3 redistribution from the apical microvilli to the base of the intermicrovillar region of the proximal tubule brush border (Zhang et al., 1999).

The chronic effect of PTH on NHE3 regulation has also been evaluated (Girardi et al., 2000; Bezerra et al., 2008). Long term inhibition of NHE3 by PTH is associated with a reduction on NHE3 protein and mRNA levels. PTH also provokes a mild inhibitory effect on NHE3 promoter that seems to be PKA-dependent (Bezerra et al., 2008).

## 2.2 Dopamine

The intrarenal dopamine natriuretic system is critical for mammalian sodium homeostasis. Numerous studies have demonstrated that dopamine remarkably increases urinary sodium excretion mainly by inhibiting tubular sodium reabsorption. The inhibitory effect of dopamine on NHE3 transport activity is mediated mainly via the dopamine D1 receptor and stimulation of adenylyl cyclase/PKA system and phospholipase C/PKC (Gomes and Soares-da-Silva, 2004). The underlying molecular mechanisms by which dopamine decreases NHE3-mediated  $\text{Na}^+/\text{H}^+$  exchange in renal proximal tubule involves increased endocytosis and is associated with increased PKA-mediated NHE3 phosphorylation (Hu et al., 2001; Bacic et al., 2003).

## 2.3 Glucagon-like peptide-1

Glucagon-like peptide-1 (GLP-1) is produced by posttranslational modification of the proglucagon gene product in the intestinal L-cells, predominantly localized in the colon and ileum (Holst, 1997; Drucker, 2005). This incretin hormone plays an important role on the maintenance of systemic glucose homeostasis by stimulating insulin secretion and improving insulin sensitivity (Drucker, 2005). Numerous reports in the literature have demonstrated that GLP-1 also exerts renoprotective actions. In this regard, continuous administration of GLP-1 induces diuresis and natriuresis in both humans (Gutzwiller et al., 2004; Gutzwiller et al., 2006) and experimental animal models (Moreno et al., 2002; Yu et al., 2003).

The molecular mechanisms underlying the renal actions of GLP-1 seems to involve increases of GFR and RPF and decrease of NHE3-mediated  $\text{Na}^+/\text{H}^+$  exchange in the renal proximal tubule (Carraro-Lacroix et al., 2009). Recent studies by our group have demonstrated that binding of GLP-1 and/or the GLP-1R agonist exendin-4 to its receptor in the renal proximal tubule activates the cAMP/PKA signaling pathway, leading, in turn, to phosphorylation of the PKA consensus sites located at the C-terminal region of the exchanger (Carraro-Lacroix

et al., 2009; Crajoinas et al., 2011). Increased NHE3 phosphorylation levels induced by GLP-1 was not accompanied by a decrease of NHE3 expression at the microvillar microdomain of the brush border, suggesting that the mechanism by which GLP-1 inhibits NHE3 activity does not involve subcellular redistribution of the exchanger between the subcompartments of the renal proximal tubule brush border.

## 2.4 Glucagon

Glucagon is a 29-amino-acid pancreatic peptide produced by the  $\alpha$ -cells present at the periphery of the islets of Langerhans (Baum et al., 1962) and its major function is the maintenance of plasma glucose homeostasis between meals and during fasting. Glucagon binding to its receptor primarily activates adenylyl cyclase and increases cAMP (Pohl et al., 1971; Rodbell et al., 1971). The tissue distribution of the glucagon receptor is broad, with higher levels of expression in liver and kidney (Svoboda et al., 1993; Dunphy et al., 1998).

In the kidney, glucagon affects renal glomerular filtration, renal blood flow, and decreases renal tubular sodium reabsorption (Pullman et al., 1967). Part of the acute natriuretic action of glucagon are mediated by inhibition of NHE3 in the renal proximal tubule via a cAMP/PKA-dependent pathway. Interestingly, *in vitro* studies using OKP cells have shown that glucagon acutely inhibits and chronically stimulates NHE3 activity (Amemiya et al., 2002).

## 2.5 Guanylins

The guanylin and uroguanylin are endogenous ligands of the *Escherichia coli* heat-stable enterotoxin (STa) receptor, guanylate cyclase C (Currie et al., 1992; Fonteles et al., 1998) and are known to be involved in a control system that regulates salt balance in response to oral salt intake. Both guanylin and uroguanylin are synthesized in the intestine and in the kidney and have already been identified in several animal species, including as mammals, fishes and birds (Forte, 2004).

The renal effects of uroguanylin are much more pronounced than the ones produced by guanylin and include natriuresis, kaliuresis, diuresis and increased excretion of cGMP (Forte et al., 1996; Greenberg et al., 1997; Fonteles et al., 1998). In the renal proximal tubule, uroguanylin significantly inhibits NHE3 transport function (Amorim et al., 2006). Ongoing studies by the Malnic laboratory have demonstrated that the mechanism by which uroguanylin inhibits NHE3 involves increased levels of NHE3 phosphorylation followed by retrieval of the exchanger from the plasma membrane (unpublished observations, Lessa LM, Girardi AC, and Malnic G). The mechanism by which NHE3 is phosphorylated by PKA in response to coupling of uroguanylin to its receptor in the renal proximal tubule possibly involves a crosstalk mechanism between cGMP and cAMP pathways.

## 3. The Na<sup>+</sup>/H<sup>+</sup> exchanger regulatory factor NHERF

Although a large number of hormones reported to affect NHE3 share the same signal pathways, the molecular mechanisms by which they regulate NHE3 may differ greatly among them. The identification of regulatory proteins that interact with NHE3 has unraveled some aspects of the molecular mechanisms underlying this transporter regulation.

The first NHE3 regulatory factor was isolated and characterized by Weinman and Shenolikar (Weinman et al., 1995; Weinman et al., 2000a; Weinman et al., 2000b). These investigators



demonstrated that the presence of this cofactor was essential for PKA-mediated inhibition of NHE3. This protein was cloned and termed NHERF-1 (Na/H exchanger regulatory factor). Subsequently, in an attempt to identify proteins that interact with NHE3, Yun and coworkers used the C-terminus of NHE3 as a bait in a yeast two-hybrid screen and isolated E3KARP (exchanger-3 kinase A regulatory protein, or NHERF-2). NHERF-1 and NHERF-2 are highly homologous proteins (52% sequence identity for the human orthologs) (Yun et al., 1997). Physical association of NHERF-1 and NHERF-2 with NHE3 has been demonstrated by binding assays using fusion proteins or by co-precipitation experiments using transfected cells overexpressing NHE3.

NHERF-1 and NHERF-2 are both members of a family of proteins that contain two tandem PDZ domains (that are conserved modules that mediate protein-protein interaction) and a C-terminal ezrin-radixin-moesin (ERM) binding domain which anchors the proteins to the actin cytoskeleton through ezrin. Lamprecht and Yun have proposed a model whereby the complex NHERF/ezrin acts as a functional AKAP (A kinase anchoring protein) for NHE3, serving as a structural link between not only NHE3 and the cytoskeleton but also between NHE3 and PKA, since ezrin is capable of binding the RII regulatory subunit of PKA (Dransfield et al., 1997; Lamprecht et al., 1998). The current model suggests that NHERF-1 is required for cAMP-dependent regulation of NHE3 and acts as an adapter to link NHE3 to ezrin, which then serves as a PKA anchoring protein. Upon activation of PKA by hormones and/or factors that increase intracellular cAMP, PKA phosphorylates serine residues in the C-terminal hydrophilic domain of NHE3 (Fig. 4). Biochemical experiments with brush border vesicles isolated from NHERF-1 knockout mice corroborate with this model (Weinman et al., 2003). These studies showed that NHERF-1 is crucial for PKA-mediated phosphorylation and inhibition of NHE3 transport activity. Moreover, NHE3 expression was not affected in these animals, showing that NHERF-1 plays an essential role on NHE3 modulation but it is not required for expression or apical targeting of the transporter in the proximal tubule (Weinman et al., 2003).

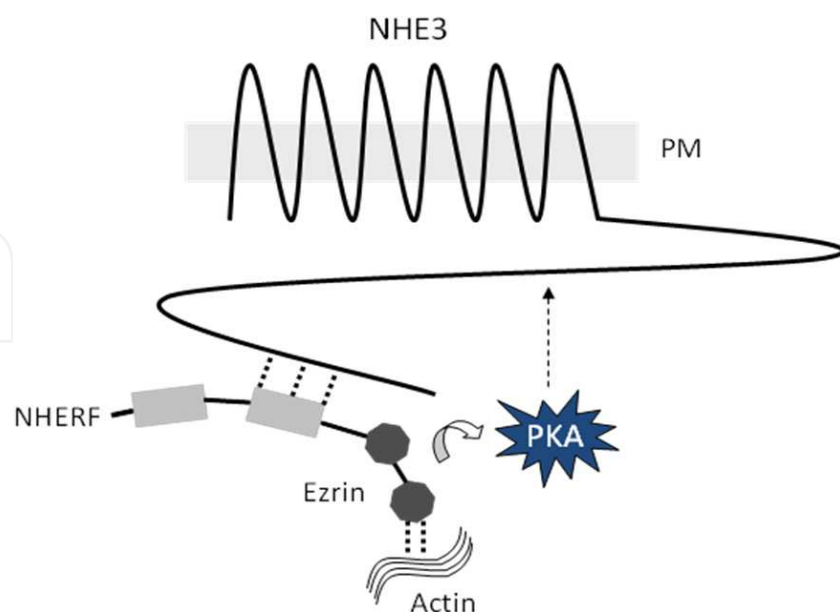


Fig. 4. Model of NHERF requirement for PKA-mediated NHE3 inhibition. NHERF facilitates cAMP-dependent regulation of NHE3 by interacting with the cytoskeleton to target PKA to phosphorylation sites within the cytoplasmic domain of NHE3.

To date, four members of the NHERF family of PDZ domain proteins have been described. These proteins bind to a variety of membrane transporters regulating their cell surface expression, protein interactions as well as the formation of signaling complexes.

#### 4. NHE3 phosphorylation at the PKA consensus sites

Moe and coworkers were the first to demonstrate that the cytoplasmic domain of NHE3 is a substrate for PKA *in vitro* (Moe et al., 1995). There are multiple putative consensus motifs for PKA on the NHE3 C-terminal region. Three of these sites (S552, S605 and S634) are highly conserved among species. Based on that, transfection studies using truncation of the NHE3 C-terminal domain and site direct mutagenesis of the above mentioned serine residues were carried out to evaluate whether PKA directly phosphorylates one or more of these consensus sites *in vivo* (Cabado et al., 1996; Kurashima et al., 1997; Zhao et al., 1999). Kurashima and coworkers have shown that both serines 605 and 634 are important for PKA-mediated inhibition of NHE3, although only serine 605 is phosphorylated *in vivo* (Kurashima et al., 1997). The studies by Zhao and colleagues confirmed that phosphorylation of serine 605 is increased by cAMP/PKA. These investigators also found that PKA directly phosphorylates serine 552 and that both 552 and 605 residues appear to be critical for inhibition of NHE3 by PKA (Zhao et al., 1999).

Years later, the Aronson laboratory generated phosphospecific antibodies directed to the PKA consensus sites S552 and S605 of rat NHE3 (Kocinsky et al., 2005). These reagents are of great value, since they have enable investigators to evaluate the phosphorylation state of these two residues in endogenous NHE3 under basal conditions, under a variety of physiological and pharmacological maneuvers and in disease states. Indeed, increments in the phosphorylation status of NHE3 at serines 552 and 605 have been shown to occur in response to dopamine (Kocinsky et al., 2005), PTH (Kocinsky et al., 2007), and GLP-1 (Crajoinas et al., 2011).

Studies by Kocinsky and colleagues have also demonstrated that serine 552 is phosphorylated to a much greater extent than serine 605 in baseline *in vivo*. Moreover, these investigators found that when NHE3 is phosphorylated at serine 552, it mainly resides at the intermicrovillar domain of the brush border (Kocinsky et al., 2005). This observation is consistent with a decrease on NHE3-mediated Na<sup>+</sup>/H<sup>+</sup> exchange in the renal proximal tubule, since within the intermicrovillar subcompartment of the brush border, this transporter must have very limited access to the tubular fluid.

The precise mechanism by which NHE3 phosphorylation leads to NHE3 inhibition remains obscure. Although phosphorylation of NHE3 at the 552 and 605 residues are necessary for the PKA-dependent inhibitory effect, phosphorylation of NHE3 at these PKA consensus sites precedes transport inhibition (Kocinsky et al., 2007) indicating that phosphorylation per se is not sufficient to inhibit NHE3 activity. The current body of data suggests that PKA phosphorylation may ultimately result in inhibition of NHE3 by modulating NHE3 subcellular trafficking, interaction with regulatory proteins, or localization within the plasma membrane.

#### 5. Pathophysiological Implications of NHE3 Phosphorylation by Protein Kinase A

As mentioned above, NHE3 is phosphorylated on serine 552 under basal conditions by the adenylyl cyclase/cAMP-activated-protein kinase A (PKA) and the endogenous levels of

phosphorylation is often affected as part of acute NHE3 regulation. Interestingly, baseline levels of NHE3 phosphorylation at this residue may also be associated with chronic regulation of NHE3 activity.

### 5.1 High Salt Diet

During high salt diet the kidneys increase sodium and volume excretion to match intake. Yang and colleagues have demonstrated that three weeks of high salt diet (4%) doubled the levels of NHE3 phosphorylation at the serine 552 (Yang et al., 2008), possibly contributing to the natriuretic effect triggered by high sodium load.

### 5.2 Hypertension

Mice lacking Na<sup>+</sup>/H<sup>+</sup> exchanger NHE3 are hypotensive and hypovolemic, underscoring the importance of the transporter on blood pressure control.

We have recently assessed *in vivo* NHE3 transport activity and defined the mechanisms underlying NHE3 regulation before and after development of hypertension in spontaneously hypertensive rats (SHR). By means of *in vivo* stationary microperfusion, we found that NHE3-mediated bicarbonate reabsorption is higher in the proximal tubule of 5-week-old pre-hypertensive spontaneously hypertensive rat (SHR) and lower in 14-week-old SHR compared to age-matched normotensive rats (Wistar Kyoto, WKY). Higher NHE3 activity in young pre-hypertensive SHR is associated with lower phosphorylation levels (serine 552) and increased NHE3 expression at the microvillar brush border. During the hypertensive stage, NHE3 was found to be mainly confined to the intermicrovillar region and the relative abundance of NHE3 phosphorylated on serine 552 is increased compared to normotensive animals (Fig. 5) (Crajoinas et al., 2010).

### 5.3 Heart failure

Heart failure (HF) is associated with sodium and water retention and extracellular volume expansion. A principal site of renal salt and water reabsorption is the proximal tubule. We therefore hypothesized that NHE3, the major apical transcellular pathway for sodium reabsorption in the proximal tubule, might be upregulated in heart failure. To test this hypothesis, we employed both stationary *in vivo* microperfusion and pH-dependent sodium uptake to verify whether NHE3 activity would be altered in the proximal tubule of an experimental model of heart failure (Antonio et al., 2009). Our data demonstrated that heart failure rats display enhanced NHE3-mediated sodium reabsorption in the proximal tubule which may contribute to extracellular volume expansion and edema. In addition to increased renal cortical NHE3 expression at both protein and mRNA levels, we have also observed that the levels of NHE3 phosphorylation at serine 552 in renal cortical membranes of heart failure rats are lower than in Sham animals. Thus, the molecular mechanisms mediating enhanced sodium reabsorption in the renal proximal tubule of heart failure rats also involves posttranslational covalent modification of NHE3 (Fig. 5) (Inoue et al., 2012).

## 6. Conclusion

PKA activation plays an important role in inhibiting the activity NHE3, the major apical transcellular pathway for sodium reabsorption in the proximal tubule. Similarly, PKA

inhibition may be involved in NHE3 stimulation, as suggested by the studies carried out in young hypertensive and in heart failure animals. The elucidation of the mechanisms by which phosphorylation of NHE3 at the PKA consensus sites leads to inhibition of NHE3-mediated Na<sup>+</sup>/H<sup>+</sup> exchange in the renal proximal tubule may also unravel important molecular underpinnings that lead to the development and/or progression of primary kidney diseases and other conditions that affect the kidneys.

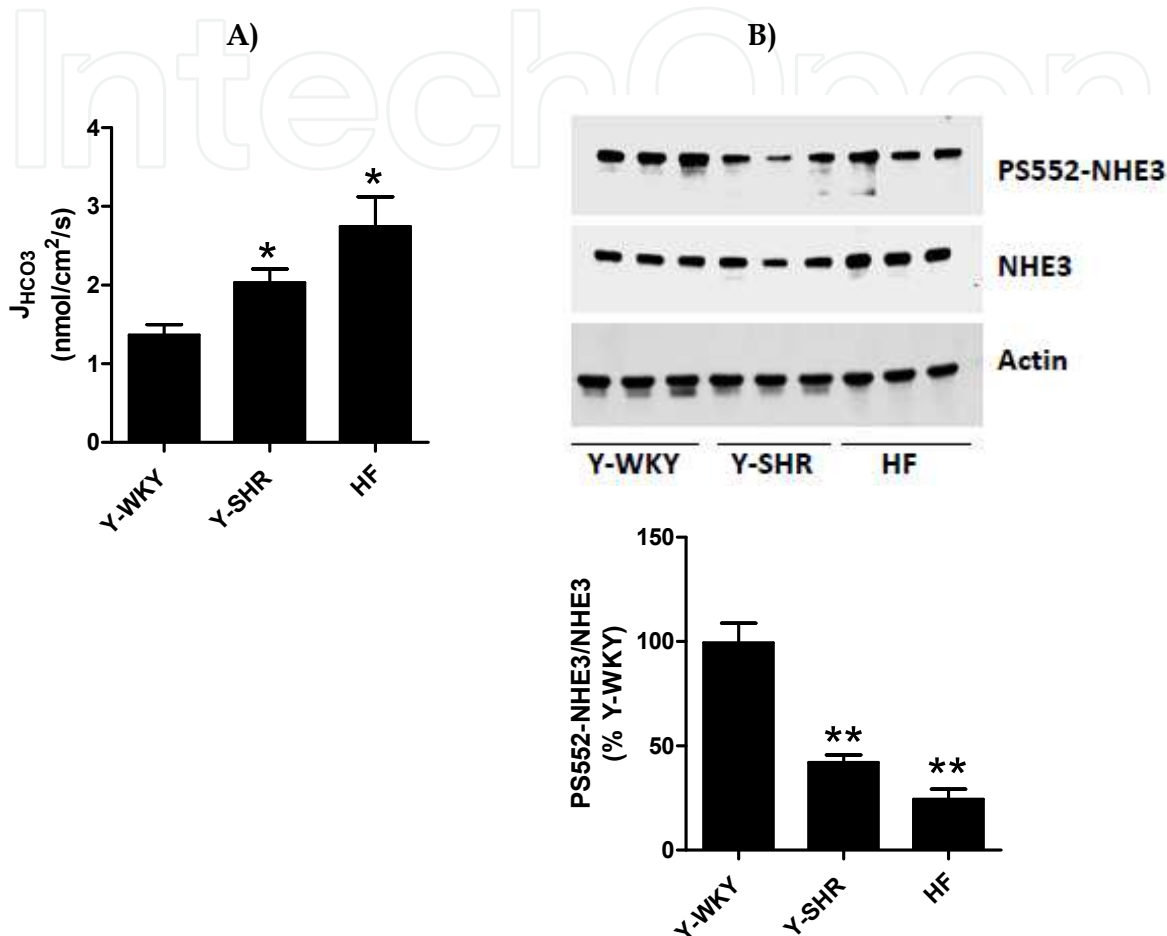


Fig. 5. NHE3 activity negatively correlates with the levels of endogenous phosphorylation of the PKA consensus site, serine 552, located at the C-terminus region of the exchanger. Adapted from (Crajinias et al., 2010; Inoue et al., 2012). **(A)** Stationary microperfusion was employed to measure NHE3-mediated bicarbonate reabsorption ( $J_{HCO_3}$ ) in the proximal tubules of 5-week-old Wistar Kyoto rats (Y-WKY,  $n = 5$  rats, 12 tubules), 5-week old SHR (Y-SHR,  $n = 5$  rats, 12 tubules, and heart failure rats (HF,  $n = 5$  rats, 15 tubules). Data are expressed as means  $\pm$  SE. \* $P < 0.05$  vs. Y-WKY. **(B) Top:** Representative immunoblot of phosphorylated and total NHE3 expression in renal cortical membranes isolated from Y-WKY, Y-SHR or HF rats. Equivalent samples (15  $\mu$ g of protein for NHE3 and 5  $\mu$ g for PS552-NHE3 and actin) of renal cortical membranes were prepared for immunoblot analysis. The membranes were incubated with monoclonal antibodies against phosphorylated NHE3 at serine 552 (PS552-NHE3 (1:1,000)), total NHE3 (1:1,000) or anti-actin (1:50,000). **Bottom: Left -** Graphical representation of the phosphorylation ratio of NHE3 at Serine 552 to total NHE3 in renal cortical membranes (PS552-NHE3/NHE3). Values are means  $\pm$  SE.  $n = 3$ /group, \*\* $P < 0.001$  vs. Y-WKY.

## 7. Acknowledgments

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## 8. References

- Alpern RJ (1990) Cell mechanisms of proximal tubule acidification. *Physiol Rev* 70:79-114.
- Amemiya M, Kusano E, Muto S, Tabei K, Ando Y, Alpern RJ and Asano Y (2002) Glucagon acutely inhibits but chronically activates Na<sup>(+)</sup>/H<sup>(+)</sup> antiporter 3 activity in OKP cells. *Exp Nephrol* 10:26-33.
- Amemiya M, Loffing J, Lotscher M, Kaissling B, Alpern RJ and Moe OW (1995) Expression of NHE-3 in the apical membrane of rat renal proximal tubule and thick ascending limb. *Kidney International* 48:1206-1215.
- Amorim JB, Musa-Aziz R, Lessa LM, Malnic G and Fonteles MC (2006) Effect of uroguanylin on potassium and bicarbonate transport in rat renal tubules. *Can J Physiol Pharmacol* 84:1003-1010.
- Antonio EL, Dos Santos AA, Araujo SR, Bocalini DS, Dos Santos L, Fenelon G, Franco MF and Tucci PJ (2009) Left ventricle radio-frequency ablation in the rat: a new model of heart failure due to myocardial infarction homogeneous in size and low in mortality. *J Card Fail* 15:540-548.
- Aronson PS (1985) Kinetic properties of the plasma membrane Na<sup>+</sup>-H<sup>+</sup> exchanger. *Annual Review of Physiology* 47:545-560.
- Aronson PS and Giebisch G (1997) Mechanisms of chloride transport in the proximal tubule. *Am J Physiol* 273:F179-192.
- Bacic D, Kaissling B, McLeroy P, Zou L, Baum M and Moe OW (2003) Dopamine acutely decreases apical membrane Na/H exchanger NHE3 protein in mouse renal proximal tubule. *Kidney Int* 64:2133-2141.
- Bank N and Aynediian HS (1976) A micropuncture study of the effect of parathyroid hormone on renal bicarbonate reabsorption. *J Clin Invest* 58:336-344.
- Baum J, Simons BE, Jr., Unger RH and Madison LL (1962) Localization of glucagon in the alpha cells in the pancreatic islet by immunofluorescent technics. *Diabetes* 11:371-374.
- Bezerra CN, Girardi AC, Carraro-Lacroix LR and Reboucas NA (2008) Mechanisms underlying the long-term regulation of NHE3 by parathyroid hormone. *Am J Physiol Renal Physiol* 294:F1232-1237.
- Biemesderfer D, Pizzonia J, Abu-Alfa A, Exner M, Reilly R, Igarashi P and Aronson PS (1993a) NHE3: a Na<sup>+</sup>/H<sup>+</sup> exchanger isoform of renal brush border. *American Journal of Physiology* 265:F736-742.
- Biemesderfer D, Pizzonia J, Abu-Alfa A, Exner M, Reilly R, Igarashi P and Aronson PS (1993b) NHE3: a Na<sup>+</sup>/H<sup>+</sup> exchanger isoform of renal brush border. *Am J Physiol* 265:F736-742.
- Biemesderfer D, Reilly RF, Exner M, Igarashi P and Aronson PS (1992) Immunocytochemical characterization of Na<sup>(+)</sup>-H<sup>+</sup> exchanger isoform NHE-1 in rabbit kidney. *American Journal of Physiology* 263:F833-840.

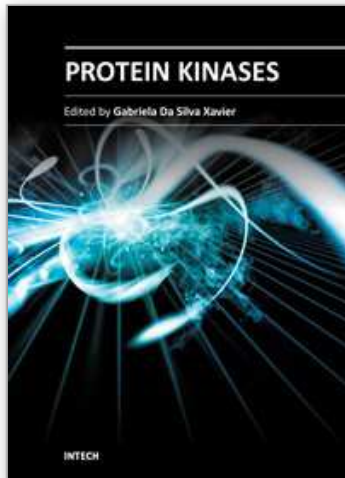
- Biemesderfer D, Rutherford PA, Nagy T, Pizzonia JH, Abu-Alfa AK and Aronson PS (1997) Monoclonal antibodies for high-resolution localization of NHE3 in adult and neonatal rat kidney. *Am J Physiol* 273:F289-299.
- Cabado AG, Yu FH, Kapus A, Lukacs G, Grinstein S and Orlowski J (1996) Distinct structural domains confer cAMP sensitivity and ATP dependence to the Na<sup>+</sup>/H<sup>+</sup> exchanger NHE3 isoform. *J Biol Chem* 271:3590-3599.
- Carraro-Lacroix LR, Malnic G and Girardi AC (2009) Regulation of Na<sup>+</sup>/H<sup>+</sup> exchanger NHE3 by glucagon-like peptide 1 receptor agonist exendin-4 in renal proximal tubule cells. *Am J Physiol Renal Physiol* 297:F1647-1655.
- Chambrey R, Achard JM, St John PL, Abrahamson DR and Warnock DG (1997) Evidence for an amiloride-insensitive Na<sup>+</sup>/H<sup>+</sup> exchanger in rat renal cortical tubules. *Am J Physiol* 273:C1064-1074.
- Chambrey R, Warnock DG, Podevin RA, Bruneval P, Mandet C, Belair MF, Bariety J and Paillard M (1998) Immunolocalization of the Na<sup>+</sup>/H<sup>+</sup> exchanger isoform NHE2 in rat kidney. *American Journal of Physiology* 275:F379-386.
- Collazo R, Fan L, Hu MC, Zhao H, Wiederkehr MR and Moe OW (2000) Acute regulation of Na<sup>+</sup>/H<sup>+</sup> exchanger NHE3 by parathyroid hormone via NHE3 phosphorylation and dynamin-dependent endocytosis. *J Biol Chem* 275:31601-31608.
- Crajoinas RO, Lessa LM, Carraro-Lacroix LR, Davel AP, Pacheco BP, Rossoni LV, Malnic G and Girardi AC (2010) Posttranslational mechanisms associated with reduced NHE3 activity in adult vs. young prehypertensive SHR. *Am J Physiol Renal Physiol* 299:F872-881.
- Crajoinas RO, Oricchio FT, Pessoa TD, Pacheco BP, Lessa LM, Malnic G and Girardi AC (2011) Mechanisms mediating the diuretic and natriuretic actions of the incretin hormone glucagon-like peptide-1. *Am J Physiol Renal Physiol* 301:F355-363.
- Currie MG, Fok KF, Kato J, Moore RJ, Hamra FK, Duffin KL and Smith CE (1992) Guanylin: an endogenous activator of intestinal guanylate cyclase. *Proc Natl Acad Sci U S A* 89:947-951.
- Dransfield DT, Yeh JL, Bradford AJ and Goldenring JR (1997) Identification and characterization of a novel A-kinase-anchoring protein (AKAP120) from rabbit gastric parietal cells. *Biochem J* 322 ( Pt 3):801-808.
- Drucker DJ (2005) Biologic actions and therapeutic potential of the proglucagon-derived peptides. *Nat Clin Pract Endocrinol Metab* 1:22-31.
- Dunphy JL, Taylor RG and Fuller PJ (1998) Tissue distribution of rat glucagon receptor and GLP-1 receptor gene expression. *Mol Cell Endocrinol* 141:179-186.
- Fan L, Wiederkehr MR, Collazo R, Wang H, Crowder LA and Moe OW (1999) Dual mechanisms of regulation of Na/H exchanger NHE-3 by parathyroid hormone in rat kidney. *J Biol Chem* 274:11289-11295.
- Felder RA, Blecher M, Calcagno PL and Jose PA (1984) Dopamine receptors in the proximal tubule of the rabbit. *Am J Physiol* 247:F499-505.
- Fonteles MC, Greenberg RN, Monteiro HS, Currie MG and Forte LR (1998) Natriuretic and kaliuretic activities of guanylin and uroguanylin in the isolated perfused rat kidney. *Am J Physiol* 275:F191-197.
- Forte LR, Fan X and Hamra FK (1996) Salt and water homeostasis: uroguanylin is a circulating peptide hormone with natriuretic activity. *Am J Kidney Dis* 28:296-304.

- Forte LR, Jr. (2004) Uroguanylin and guanylin peptides: pharmacology and experimental therapeutics. *Pharmacol Ther* 104:137-162.
- Girardi AC, Titan SM, Malnic G and Reboucas NA (2000) Chronic effect of parathyroid hormone on NHE3 expression in rat renal proximal tubules. *Kidney Int* 58:1623-1631.
- Gomes P and Soares-da-Silva P (2004) Dopamine acutely decreases type 3 Na<sup>(+)</sup>/H<sup>(+)</sup> exchanger activity in renal OK cells through the activation of protein kinases A and C signalling cascades. *Eur J Pharmacol* 488:51-59.
- Greenberg RN, Hill M, Crytzer J, Krause WJ, Eber SL, Hamra FK and Forte LR (1997) Comparison of effects of uroguanylin, guanylin, and Escherichia coli heat-stable enterotoxin STa in mouse intestine and kidney: evidence that uroguanylin is an intestinal natriuretic hormone. *J Investig Med* 45:276-282.
- Holst JJ (1997) Enteroglucagon. *Annu Rev Physiol* 59:257-271.
- Hu MC, Fan L, Crowder LA, Karim-Jimenez Z, Murer H and Moe OW (2001) Dopamine acutely stimulates Na<sup>+</sup>/H<sup>+</sup> exchanger (NHE3) endocytosis via clathrin-coated vesicles: dependence on protein kinase A-mediated NHE3 phosphorylation. *J Biol Chem* 276:26906-26915.
- Inoue BH, Dos Santos L, Pessoa TD, Antonio EL, Pacheco BP, Savignano FA, Carraro-Lacroix LR, Tucci PJ, Malnic G and Girardi AC (2012) Increased NHE3 abundance and transport activity in renal proximal tubule of rats with heart failure. *Am J Physiol Regul Integr Comp Physiol* 302:R166-174.
- Kocinsky HS, Dynia DW, Wang T and Aronson PS (2007) NHE3 phosphorylation at serines 552 and 605 does not directly affect NHE3 activity. *Am J Physiol Renal Physiol* 293:F212-218.
- Kocinsky HS, Girardi AC, Biemesderfer D, Nguyen T, Mentone S, Orlowski J and Aronson PS (2005) Use of phospho-specific antibodies to determine the phosphorylation of endogenous Na<sup>+</sup>/H<sup>+</sup> exchanger NHE3 at PKA consensus sites. *Am J Physiol Renal Physiol* 289:F249-258.
- Kurashima K, Yu FH, Cabado AG, Szabo EZ, Grinstein S and Orlowski J (1997) Identification of sites required for down-regulation of Na<sup>+</sup>/H<sup>+</sup> exchanger NHE3 activity by cAMP-dependent protein kinase. phosphorylation-dependent and -independent mechanisms. *J Biol Chem* 272:28672-28679.
- Lamprecht G, Weinman EJ and Yun CH (1998) The role of NHERF and E3KARP in the cAMP-mediated inhibition of NHE3. *J Biol Chem* 273:29972-29978.
- Ledoussal C, Lorenz JN, Nieman ML, Soleimani M, Schultheis PJ and Shull GE (2001) Renal salt wasting in mice lacking NHE3 Na<sup>+</sup>/H<sup>+</sup> exchanger but not in mice lacking NHE2. *Am J Physiol Renal Physiol* 281:F718-727.
- Lorenz JN, Schultheis PJ, Traynor T, Shull GE and Schnermann J (1999) Micropuncture analysis of single-nephron function in NHE3-deficient mice. *Am J Physiol* 277:F447-453.
- Lutken SC, Kim SW, Jonassen T, Marples D, Knepper MA, Kwon TH, Frokiaer J and Nielsen S (2009) Changes of renal AQP2, ENaC, and NHE3 in experimentally induced heart failure: response to angiotensin II AT1 receptor blockade. *Am J Physiol Renal Physiol* 297:F1678-1688.

- Magyar CE, Zhang Y, Holstein-Rathlou NH and McDonough AA (2000) Proximal tubule Na transporter responses are the same during acute and chronic hypertension. *Am J Physiol Renal Physiol* 279:F358-369.
- Marks J, Debnam ES, Dashwood MR, Srai SK and Unwin RJ (2003) Detection of glucagon receptor mRNA in the rat proximal tubule: potential role for glucagon in the control of renal glucose transport. *Clin Sci (Lond)* 104:253-258.
- Moe OW, Amemiya M and Yamaji Y (1995) Activation of protein kinase A acutely inhibits and phosphorylates Na/H exchanger NHE-3. *J Clin Invest* 96:2187-2194.
- Moreno C, Mistry M and Roman RJ (2002) Renal effects of glucagon-like peptide in rats. *Eur J Pharmacol* 434:163-167.
- Mrkic B, Forgo J, Murer H and Helmle-Kolb C (1992) Apical and basolateral Na/H exchange in cultured murine proximal tubule cells (MCT): effect of parathyroid hormone (PTH). *J Membr Biol* 130:205-217.
- Mrkic B, Tse CM, Forgo J, Helmle-Kolb C, Donowitz M and Murer H (1993) Identification of PTH-responsive Na/H-exchanger isoforms in a rabbit proximal tubule cell line (RKPC-2). *Pflugers Arch* 424:377-384.
- Muff R, Fischer JA, Biber J and Murer H (1992) Parathyroid hormone receptors in control of proximal tubule function. *Annu Rev Physiol* 54:67-79.
- Panico C, Luo Z, Damiano S, Artigiano F, Gill P and Welch WJ (2009) Renal proximal tubular reabsorption is reduced in adult spontaneously hypertensive rats: roles of superoxide and Na<sup>+</sup>/H<sup>+</sup> exchanger 3. *Hypertension* 54:1291-1297.
- Pohl SL, Birnbaumer L and Rodbell M (1971) The glucagon-sensitive adenylyl cyclase system in plasma membranes of rat liver. I. Properties. *J Biol Chem* 246:1849-1856.
- Pollock AS, Warnock DG and Strewler GJ (1986) Parathyroid hormone inhibition of Na<sup>+</sup>-H<sup>+</sup> antiporter activity in a cultured renal cell line. *Am J Physiol* 250:F217-225.
- Pouyssegur J (1994) Molecular biology and hormonal regulation of vertebrate Na<sup>+</sup>/H<sup>+</sup> exchanger isoforms. *Renal Physiology & Biochemistry* 17:190-193.
- Pullman TN, Lavender AR and Aho I (1967) Direct effects of glucagon on renal hemodynamics and excretion of inorganic ions. *Metabolism* 16:358-373.
- Rodbell M, Birnbaumer L, Pohl SL and Krans HM (1971) The glucagon-sensitive adenylyl cyclase system in plasma membranes of rat liver. V. An obligatory role of guanylnucleotides in glucagon action. *J Biol Chem* 246:1877-1882.
- Schlatter P, Beglinger C, Drewe J and Gutmann H (2007) Glucagon-like peptide 1 receptor expression in primary porcine proximal tubular cells. *Regul Pept* 141:120-128.
- Schultheis PJ, Clarke LL, Meneton P, Miller ML, Soleimani M, Gawenis LR, Riddle TM, Duffy JJ, Doetschman T, Wang T, Giebisch G, Aronson PS, Lorenz JN and Shull GE (1998) Renal and intestinal absorptive defects in mice lacking the NHE3 Na<sup>+</sup>/H<sup>+</sup> exchanger. *Nat Genet* 19:282-285.
- Svoboda M, Ciccarelli E, Tastenoy M, Robberecht P and Christophe J (1993) A cDNA construct allowing the expression of rat hepatic glucagon receptors. *Biochem Biophys Res Commun* 192:135-142.
- Vallon V, Schwark JR, Richter K and Hropot M (2000) Role of Na(+)/H(+) exchanger NHE3 in nephron function: micropuncture studies with S3226, an inhibitor of NHE3. *Am J Physiol Renal Physiol* 278:F375-379.



- Wang T, Yang CL, Abbiati T, Schultheis PJ, Shull GE, Giebisch G and Aronson PS (1999) Mechanism of proximal tubule bicarbonate absorption in NHE3 null mice. *Am J Physiol* 277:F298-302.
- Warnock DG and Yee VJ (1981) Chloride uptake by brush border membrane vesicles isolated from rabbit renal cortex. Coupling to proton gradients and K<sup>+</sup> diffusion potentials. *J Clin Invest* 67:103-115.
- Weinman EJ, Minkoff C and Shenolikar S (2000a) Signal complex regulation of renal transport proteins: NHERF and regulation of NHE3 by PKA. *Am J Physiol Renal Physiol* 279:F393-399.
- Weinman EJ, Steplock D, Donowitz M and Shenolikar S (2000b) NHERF associations with sodium-hydrogen exchanger isoform 3 (NHE3) and ezrin are essential for cAMP-mediated phosphorylation and inhibition of NHE3. *Biochemistry* 39:6123-6129.
- Weinman EJ, Steplock D and Shenolikar S (2003) NHERF-1 uniquely transduces the cAMP signals that inhibit sodium-hydrogen exchange in mouse renal apical membranes. *FEBS Lett* 536:141-144.
- Weinman EJ, Steplock D, Wang Y and Shenolikar S (1995) Characterization of a protein cofactor that mediates protein kinase A regulation of the renal brush border membrane Na<sup>(+)</sup>-H<sup>+</sup> exchanger. *J Clin Invest* 95:2143-2149.
- Yang LE, Sandberg MB, Can AD, Pihakaski-Maunsbach K and McDonough AA (2008) Effects of dietary salt on renal Na<sup>+</sup> transporter subcellular distribution, abundance, and phosphorylation status. *Am J Physiol Renal Physiol* 295:F1003-1016.
- Yu M, Moreno C, Hoagland KM, Dahly A, Ditter K, Mistry M and Roman RJ (2003) Antihypertensive effect of glucagon-like peptide 1 in Dahl salt-sensitive rats. *J Hypertens* 21:1125-1135.
- Yun CH, Oh S, Zizak M, Steplock D, Tsao S, Tse CM, Weinman EJ and Donowitz M (1997) cAMP-mediated inhibition of the epithelial brush border Na<sup>+</sup>/H<sup>+</sup> exchanger, NHE3, requires an associated regulatory protein. *Proc Natl Acad Sci U S A* 94:3010-3015.
- Zhang Y, Norian JM, Magyar CE, Holstein-Rathlou NH, Mircheff AK and McDonough AA (1999) In vivo PTH provokes apical NHE3 and NaPi2 redistribution and Na-K-ATPase inhibition. *Am J Physiol* 276:F711-719.
- Zhao H, Wiederkehr MR, Fan L, Collazo RL, Crowder LA and Moe OW (1999) Acute inhibition of Na/H exchanger NHE-3 by cAMP. Role of protein kinase a and NHE-3 phosphoserines 552 and 605. *J Biol Chem* 274:3978-3987.



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Proteins are the work horses of the cell. As regulators of protein function, protein kinases are involved in the control of cellular functions via intricate signalling pathways, allowing for fine tuning of physiological functions. This book is a collaborative effort, with contribution from experts in their respective fields, reflecting the spirit of collaboration - across disciplines and borders - that exists in modern science. Here, we review the existing literature and, on occasions, provide novel data on the function of protein kinases in various systems. We also discuss the implications of these findings in the context of disease, treatment, and drug development.

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