

ISN Forefronts Symposium 2015: Maintaining Balance Under Pressure—Hypertension and the Proximal Tubule



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Renal control of effective circulating volume (ECV) is key for circulatory performance. When renal sodium excretion is inadequate, blood pressure rises and serves as a homeostatic signal to drive natriuresis to re-establish ECV. Recognizing that hypertension involves both renal and vascular dysfunction, this report concerns proximal tubule sodium hydrogen exchanger 3 (NHE3) regulation during acute and chronic hypertension. NHE3 is distributed in tall microvilli (MV) in the proximal tubule, where it reabsorbs a significant fraction of the filtered sodium. NHE3 redistributes, in the plane of the MV membrane, between the MV body, where NHE3 is active, and the MV base, where NHE3 is less active. A high-salt diet and acute hypertension both retract NHE3 to the base and reduce proximal tubule sodium reabsorption independent of a change in abundance. The renin angiotensin system provokes NHE3 redistribution independent of blood pressure: The angiotensin-converting enzyme (ACE) inhibitor captopril redistributes NHE3 to the base and subsequent angiotensin II (AngII) infusion returns NHE3 to the body of the MV and restores reabsorption. Chronic AngII infusion presents simultaneous AngII stimulation and hypertension; that is, NHE3 remains in the body of the MV, due to the high local AngII level and inflammation, and exhibits a compensatory decrease in abundance driven by the hypertension. Genetically modified mice with blunted hypertensive responses to chronic AngII infusion (due to lack of the proximal tubule AngII receptors interleukin-17A or interferon- γ expression) exhibit reduced local AngII accumulation and inflammation and larger decreases in NHE3 abundance, which improves the pressure natriuresis response and reduces the need for elevated blood pressure to facilitate circulating volume balance.

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In 1909, Ernest Starling published his lectures in *The Fluids of the Body*.¹ In this monograph, Starling outlines his thoughts on the relationship between body fluid balance, blood circulation, central venous pressure, and cardiac performance. Importantly, renal control of body fluid balance is viewed as key for circulatory and cardiac performance:¹

Change in the blood flow through the kidney may bring about alterations in the flow of urine quite irrespective of the composition of the blood or of the tissues. The occurrence of these two classes of phenomena seems to be determined by the fact that the kidney is a dual organ, and that while one part of it acts, so to speak, passively in

response to force impressed upon it from without, another part, endowed with sensibility, reacts to external forces in a direction which may be opposed to these forces, but is in all cases the appropriate one for the welfare of the whole organism.

In 1963, Borst and Borst-de Geus² discussed hypertension in light of Starling's theory of circulatory homeostasis and postulated that blood pressure rises as a homeostatic reaction to deficient sodium excretion; that is, pressure rises to reestablish sodium balance (at the expense of persistently elevated blood pressure).² This response, known as pressure natriuresis, has been reviewed from various perspectives by many investigators.^{3,4–7} Figure 1 shows central blood pressure as a function of the ECV and cardiac output as well as the kidneys' regulation of sodium chloride and water reabsorption. A kidney's decision to excrete sodium chloride and water is a function of mediators and controls, both extrarenal and intrarenal, that affect the

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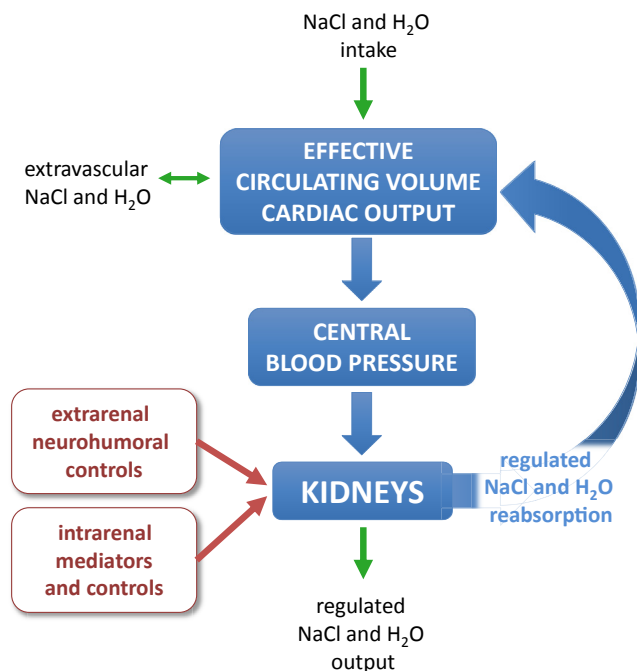


Figure 1. Central blood pressure shown as a function of the effective circulating volume and cardiac output as well as the kidneys' regulation of sodium chloride (NaCl) and water (H₂O) reabsorption, adapted from Starling¹ and Borst and Borst-De Geus.² A kidney's willingness to excrete NaCl and water when blood pressure rises is a key controller of the effective circulating volume and blood pressure. This article examines factors, both extrarenal and intrarenal, that reduce this willingness in the proximal tubule (and mechanisms involved), leading to an elevation in blood pressure and activation of pressure natriuresis mechanisms in the proximal tubule that contribute to maintenance of effective circulating volume homeostasis.

rise in blood pressure, as well as the extravascular storage of sodium (discussed in an accompanying report in this series).⁸

Hypertension is the leading cause of stroke and cardiovascular diseases affecting 30% of the adult population in Western cultures.⁹ Blood pressure can be elevated by vasoconstriction or by increasing ECV. Excess sodium reabsorption raises ECV and blood pressure, yet, according to Guyton,³ kidneys have the capacity via pressure natriuresis to excrete enough sodium and volume to normalize blood pressure in the face of expanded ECV. Hypertension was classically viewed as a failure of pressure natriuresis; however, a recent discussion of the role of kidneys in the pathogenesis of hypertension¹⁰ concluded that for hypertension to become chronic there must be impairment of both renal output of salt and water as well as dysfunction of peripheral vascular tone; for example, a failure of peripheral vasodilation due to arterial stiffness. Support for the latter is provided by recent studies illustrating a positive feedback loop wherein arterial stiffening leads to more arterial stiffening.¹¹

Although appreciating these complex interactions of body fluids, cardiac output, vascular stiffness, and blood pressure, this report will focus on the regulation of the renal proximal tubule NHE3 as a case-in-point mediator of the pressure natriuresis response, specifically regulation of NHE3 trafficking and abundance, our understanding of how renal dysfunction resets NHE3 regulation to higher pressures, and strategies that may be exploited to improve pressure natriuresis.

Proximal Tubule NHE3 Regulated by Redistribution Within the MV

As reviewed by Palmer and Schnermann,¹² the proximal tubule reabsorbs two-thirds of the salt and water filtered at the glomerulus (120 ml/min) and NHE3 is the main sodium transporter driving transcellular reabsorption in this region. The proximal tubule is a leaky epithelium well built to reabsorb the ~80 ml/min filtrate. As illustrated in the cross-section of the electron micrograph in Figure 2, the proximal tubule has an apical pole covered with a tall brush border of MV each scaffolded by an actin filament core bundled by villin. This specialization increases the surface area for reabsorption more than 30-fold.¹³ The apical MV contain water channels as well as many different transporters to reabsorb cations, anions, and substrates from the filtrate. Importantly, a significant fraction of the filtered salt and water is reabsorbed via a paracellular route by claudins.¹⁴

Membrane transporters and channels can be regulated by trafficking between plasma membrane and intracellular membranes, altered total pool size, covalent modifications such as cleavage or phosphorylation, or protein-protein interaction. Once NHE3 is localized to the proximal tubule MV, there is scant *in vivo* evidence for regulated trafficking between MV and intracellular pools. Rather, NHE3, localized to ordered lipid domains (rafts) in the MV, redistribute between the body and the base of the MV, moving in the plane of the microvillar membranes, likely driven by the atypical molecular motor myosin VI.^{15–17} This redistribution from one location to another, rather than degradation and synthesis, facilitates rapid continuous adaptation to changing salt intake, ECV, and/or blood pressure. Figure 2 illustrates the simple case of NHE3 regulation in the transition between normal and high-salt diets in the absence of any change in blood pressure.¹⁸ Figure 2a illustrates that this natriuresis occurs without any change in NHE3 total abundance. Figure 2b shows cross-sections of proximal tubules in a model and in an electron micrograph illustrating organization of dense apical MV. NHE3 redistribution along the proximal tubule MV is detected by colabeling the actin bundling protein villin (red V) and NHE3

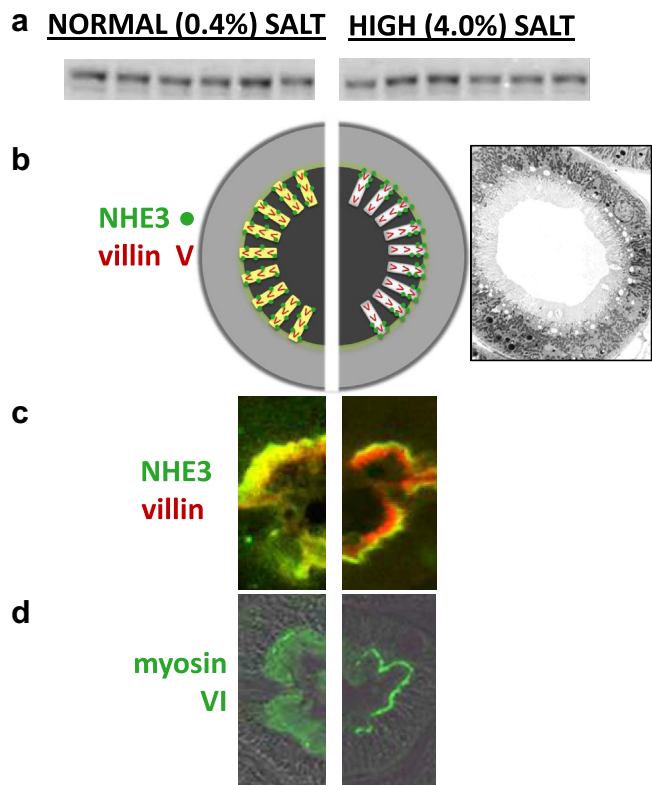


Figure 2. Elevating salt intake from 0.4% to 4% does not change the total abundance of proximal tubule sodium hydrogen exchanger 3 (NHE3), but redistributes NHE3 to the base of the proximal tubule microvilli. In salt-resistant animals this occurs without an elevation in blood pressure. (a) Immunoblots of renal cortical homogenates from rats fed 0.4% or 4% salt diet.¹⁸ (b) Cross-section of proximal tubule shown in a simple model and electron micrograph illustrating dense apical microvilli. NHE3 redistribution along the proximal tubule microvilli is detected by colabeling the actin bundling protein villin (red V) and NHE3 (green circle) with specific antibodies.¹⁸ Left half of the proximal tubule model represents the proximal tubule at a normal-salt diet with the villin and NHE3 colocalized, yielding microvilli stained yellow. The right half of the proximal tubule represents the proximal tubule during a high-salt diet with NHE3 retracted to the base of the microvilli exposing red V in the body of the villi and green/yellow at the base of the microvilli. (c) Colabeling of the proximal tubule from rats fed 0.4% sodium chloride (left half-tubule) with both NHE3 and villin in the body of the microvilli, and 4.0% sodium chloride (right half-tubule) with NHE3 concentrated at the base of the microvilli. (d) Myosin VI, an atypical molecular motor implicated in the redistribution of NHE3 and sodium phosphate within the plane of the microvillar membrane,^{17,19} is located in the body of the microvilli with a 0.4% sodium chloride diet and redistributes to the base of the microvilli during a 4% sodium chloride diet.

(green circle) with specific antibodies.¹⁸ The left half of the model represents a proximal tubule from a normal-salt-diet-fed rat with villin and NHE3 colocalized in the MV, yielding a yellow stain. The right half of the proximal tubule model represents a proximal tubule from a high-salt-diet-fed rat wherein NHE3 is retracted to the base of the MV, exposing a red V in body of the villi and green/yellow at the base of the MV. Figure 2c demonstrates NHE3 in the body of the proximal tubule

MV in rats fed 0.4% sodium chloride (left half-tubule), and to the base of the MV, in rats fed 4.0% sodium chloride (right half-tubule). Myosin VI, an atypical molecular motor implicated in the redistribution of NHE3 and sodium phosphate transporter within the plane of the microvillar membrane,^{17,19} also redistributes from the body to the base of the MV during high-salt diet, presumably driving the NHE3 (Figure 2d). As discussed below, when NHE3 is clustered at the base of the MV, its activity is predicted to be inhibited by unfavorable pH gradients.²⁰ Recent studies have shown that when the excretory function of the kidney is chronically impaired by inhibiting nitric oxide synthase activity, the resultant renal inflammation blunts the depression in sodium transport during high-salt diet, leading to a rise in blood pressure; that is, the renal dysfunction leads to salt-sensitive hypertension.²¹

Evidence for a Role of Proximal Tubule in Acute Pressure Diuresis

The classic acute pressure natriuretic protocol developed by Roman and Cowley²² and a typical response is illustrated in Figure 3a: In inactin-anesthetized male rats, raising mean arterial blood pressure from 87 to 130 mm Hg rapidly increases urine output more than 10-fold.^{23,24} Because the pressure–natriuresis response is very large and rapid, the proximal tubule was identified as a good candidate region for natriuresis—it reabsorbs the bulk of the filtered load. In the mid-1980s, Chou and Marsh^{25,26} developed a video-densitometric approach to analyze tubular flow in real time and found that acutely raising blood pressure rapidly increased end proximal tubule flow rate by 50%. Because they also demonstrated this occurred without appreciable changes in glomerular filtration rate (GFR) or renal blood flow (due to autoregulation), they concluded that proximal tubule sodium transport was inhibited during acute hypertension, and that this response contributed not only to pressure diuresis, but also to the autoregulation of GFR and renal blood flow (mediated by increasing salt delivery to the macula densa). This response confirmed Starling's assertions that “the mechanisms, which determine the adaptation of the organism to changes in the total volume of its fluid content, must come into play with every rise or fall in the general blood pressure.”¹

NHE3 Regulated by Redistribution Within the MV During Acute Hypertension

The renal natriuretic and diuretic responses to acute or chronic increases in blood pressure are referred to very generally as pressure natriuresis. The responses to acute hypertension in the proximal tubule (Figure 3)

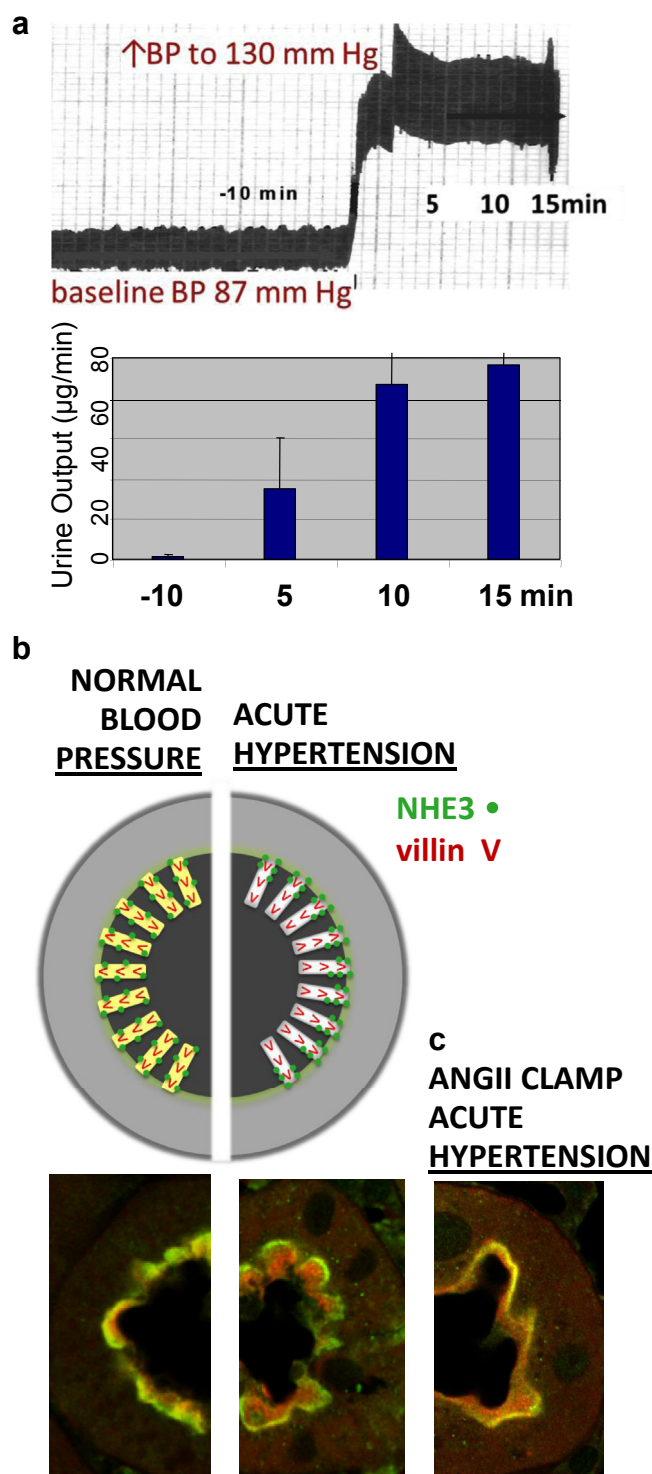


Figure 3. Acute hypertension produced by raising total peripheral resistance rapidly increases urine salt and volume output (pressure natriuresis/diuresis) and redistributes the sodium hydrogen exchanger 3 (NHE3) to the base of the proximal tubule microvilli. (a) In inactin-anesthetized male rats, raising mean atrial blood pressure (BP) from 87 to 130 mm Hg according to the protocol of Roman and Cowley²² rapidly increases urine output more than 10-fold.²³ (b) The cross-section model of proximal tubule as described in Figure 2. Colabeling of the proximal tubule from rats at baseline BP (left half-tubule) with both NHE3 and villin in the body of microvilli, and at elevated BP (right half-tubule) with NHE3 concentrated at the base of the microvilli.¹⁶ (c) When angiotensin II (AngII) levels are clamped

are analogous to those observed during a high-salt diet (Figure 2). The pressure–natriuretic signals, discussed below, provoke the dynamic redistribution of apical transporters, including NHE3 and sodium phosphate cotransporter II (NaPiII),¹⁶ driven by molecular motors (eg, myosin VI and IIA) and cytoskeleton-associated proteins, to the base of the proximal tubule MV (Figure 3b).^{17,19} The lipid raft-associated NHE3 remains at the base¹⁵ and the nonraft-associated NaPiII is endocytosed, culminating in decreased sodium transport activity and increased proximal tubule flow rate.^{7,27,28} Recently, with Brasen *et al.*,²⁰ we visualized the hypertension-stimulated redistribution of NHE3 to the base of the MV *in vivo* using 2-photon microscopy with the pH indicator BCECF. Mathematic modeling of this redistribution suggests that NHE3 clustering produces unfavorable pH microdomains near the bottom of the brush border sufficient to inhibit NHE3 activity.²⁰ This conclusion helps to explain how NHE3 redistribution can contribute to natriuretic responses during a high-salt diet and acute hypertension.

The signaling that provokes the rapid decrease in proximal tubule sodium and volume reabsorption, and retraction of NHE3 to the base of the MV in the face of autoregulated renal blood flow and GFR appears to involve many layers of regulation by both intrinsic and extrinsic factors. Summarizing the findings of multiple labs, it appears that the initial natriuretic response is driven by rapid local generation of 20-hydroxyeicosatetraenoic acid and nitric oxide (NO),^{28–30} (perhaps involving nonautoregulating vasculature that senses the hypertension), and that the response in the proximal tubule involves the production of cyclic guanosine monophosphate,³¹ which plays a role in depressing sodium transport. We found that clamping AngII levels at a nonpressor level by coinfusion of both the ACE inhibitor captopril and AngII before the acute hypertension protocol significantly blunted the pressure diuresis as well as the redistribution of NHE3 to the base of the MV (Figure 3c).^{32,33} The decrease in AngII is key to not only allow NHE3 redistribution to the base of the MV, but also to sustain the response by reducing sodium transport in AngII-sensitive regions all along the nephron, including the distal tubule.³⁴ Not the focus of this review, but important to discuss in light of

← by preinfusion with an angiotensin-converting enzyme inhibitor to prevent AngII production along with a constant infusion of AngII to maintain baseline BP, the redistribution of NHE3 to the base of the microvilli is significantly blunted.³² The findings suggest that a drop in local AngII may contribute to the NHE3 redistribution during acute hypertension, and that high local AngII can blunt pressure natriuresis.

signaling, is the fact that the medullary loop of Henle also clearly participates in pressure natriuresis during hypertension: medullary blood flow, NO, and reactive oxygen species participate as signals.^{35,36} A recent report from Crowley *et al.*³⁷ suggests that inflammatory accumulation of interleukin-1 during hypertension activates loop of Henle Na⁺-K⁺- 2Cl⁻ cotransporter (NKCC2), which blunts the natriuresis and raises blood pressure. Eliminating interleukin-1 limits the blood pressure elevation by reducing NKCC2 sodium reabsorption. This study provides another example of how injury signals contribute to hypertension by blunting pressure natriuresis. In summary, regarding natriuretic signaling during hypertension, there is consensus that multiple signals have the potential to act all along the nephron; thus, the pressure natriuresis response is the sum of the prevailing natriuretic and antinatriuretic influences.

Renin Angiotensin System Regulates Proximal Tubule NHE3 Distribution and Abundance

The renin angiotensin aldosterone system is the most powerful controller of ECV and blood pressure, as recently reviewed by Rossier.³⁸ AngII increases sodium reabsorption in the proximal tubule mediated by AT1 receptors. Building on the findings of the suppression of pressure natriuresis during the AngII clamp, we investigated the acute effects of adding or inhibiting AngII without changing blood pressure. Because ACE inhibitors are among the most popular drugs prescribed to lower blood pressure and slow the progression of renal and heart disease, it is key to understand how they regulate the proximal tubule NHE3. We addressed this issue by merging physiology and proteomics.³⁹ Leong *et al.*³⁹ identified a dose of the ACE inhibitor captopril that, when infused for 20 minutes into anesthetized rats, did not change blood pressure or GFR, but did significantly increase urine output (12 µg/min). With the Yip lab, they demonstrated that this dose rapidly increased proximal tubule flow rate, evidence for ACE inhibitor suppression of proximal tubule sodium reabsorption; **Figure 4b** shows that NHE3 is retracted to the microvillar base after 20 minutes of ACE inhibitor treatment, evidence that basal AngII is responsible, at least in part, for the location of NHE3 within the MV at baseline.³⁹ With the Klein lab, Leong *et al.*³⁹ applied a limited proteomic approach and discovered that other brush border proteins that redistribute with captopril include myosin VI, dipeptidyl peptidase VI, NHERF-1, ezrin, megalin, vacuolar H⁺-ATPase, aminopeptidase N, and clathrin.

Starting with ACE inhibitor-infused rats, Riquier-Brison *et al.*⁴⁰ determined the effects of acute AngII infused at a rate that did not alter blood pressure for

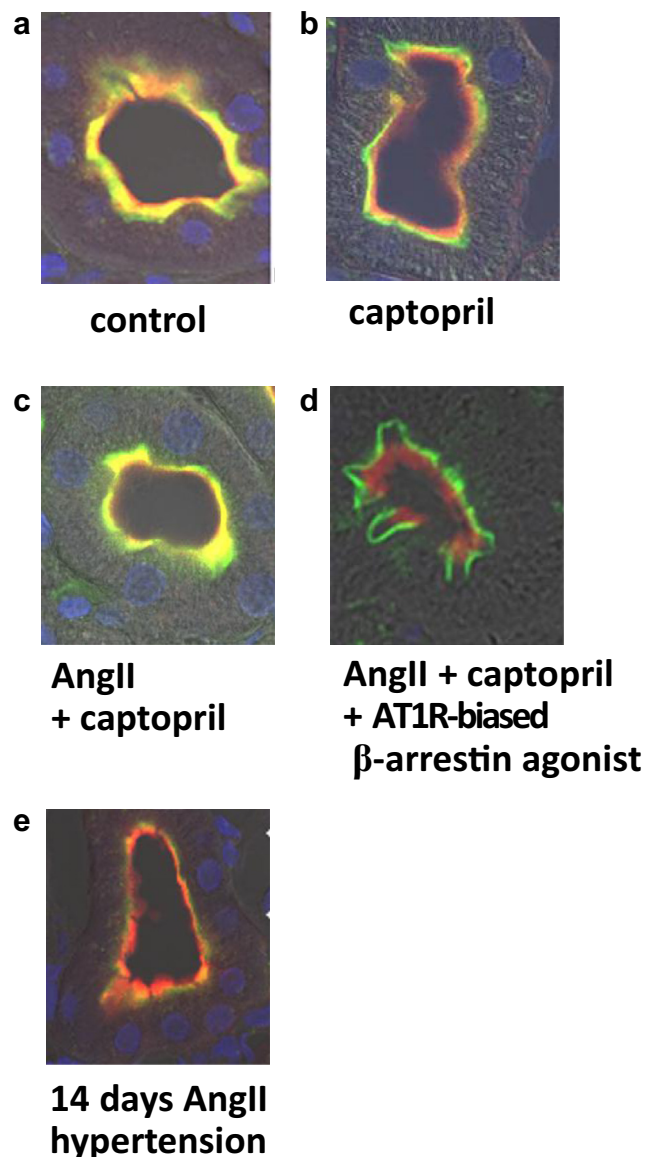


Figure 4. Angiotensin II (AngII) regulates sodium hydrogen exchanger 3 (NHE3) distribution in the proximal tubule microvilli. Renal cortex examined by colabeling NHE3 (green) and villin (red) with specific antibodies as described in **Figure 1**. (a) NHE3 in body of the microvilli in an inactin-anesthetized rat at baseline. (b) Infusing captopril for 20 minutes at a dose that does not lower blood pressure (12 µg/min) redistributes NHE3 to the base of the microvilli.³⁹ (c) Infusing captopril for 20 minutes followed by AngII infusion for 20 minutes (20 ng/kg/min), at doses that do not change blood pressure, moves NHE3 from the base to the body of the microvilli.⁴⁰ (d) Coinfusing a β-arrestin-biased AngII receptor-(AT1R) specific agonist along with captopril for 20 minutes (TRV120023) moved NHE3 to the base of the microvilli and blocked the effects of subsequent AngII infusion.⁴¹ (e) Infusion of AngII into rats for 14 days (400 ng/kg/min) raised the mean arterial pressure from 120 to 160 mm Hg and decreases the total abundance of renal cortical NHE3 by 20%, yet despite the hypertension NHE3 is retained in the body of the microvilli.³⁵ Images in panels a and e were collected with the same settings, demonstrating that the ratio of NHE3 to the villin signal has decreased during AngII.

another 20 minutes (20 ng/kg/min) and found that the NHE3 completely returned to the body of the MV (AngII + captopril) (**Figure 4c**). Leong *et al.*³⁹ previously

reported that AngII + captopril infusion also decreased proximal tubule flow rate, providing evidence that the NHE3 redistribution is antinatriuretic. In collaboration with Carneiro de Morais *et al.*,⁴¹ we recently investigated the effects of a β -arrestin-biased AT1 receptor (AT1R) agonist (TRV120023) on proximal tubule NHE3. Stimulating β -arrestin-biased pathways promotes G-protein receptor internalization and desensitization and can activate G-protein independent responses.⁴² Perfusing proximal tubules *in vivo* with the TRV compound reduced bicarbonate reabsorption, a surrogate measure of NHE3 transport, and also moved NHE3 to the bottom of the MV.⁴¹ TRV can also prevent the effects of AngII activation: Coinfusing the TRV compound with ACE inhibitors for 20 minutes blocks the effects of subsequent 20-minute AngII infusion and redistributes the NHE3 to the base (Figure 4d).

Experimental AngII hypertension involves infusing AngII continuously at a dose that is initially subpressor but eventually provokes hypertension. Using immunoblots of the renal cortex, we determined in rats that after 3 days of AngII infusion (200 ng/kg/min), before blood pressure increase, the total abundance of cortical NHE3 increases around 50% above baseline,⁴³ and then by 14 days of AngII infusion (at 400 ng/kg/min), when blood pressure is chronically elevated to 160 mm Hg, the NHE3 abundance is depressed to 20% below baseline.³⁵ Figure 4e shows a proximal tubule from a 14-day AngII-infused rat with hypertension in which NHE3 is retained in the body of the MV.³⁵ Images in Figure 4a and e were collected with the same settings, demonstrating that the ratio of NHE3 to villin signal decreased in the MV during AngII hypertension. Overall, these findings suggest that AngII “fixes” NHE3 in the MV and blunts redistribution, both acutely (Figure 3c) and chronically (Figure 4e). In lieu of redistribution, a compensatory decrease in NHE3 abundance in the MV becomes evident as blood pressure increases. The biphasic effects of AngII on proximal tubule reabsorption reported in rodents⁴⁴ have led to the suggestion that the decreased NHE3 abundance may be due to high-dose AngII inhibition of NHE3, rather than hypertension. However, this inhibitory effect is observed when AngII was directly applied to tubules at doses of ($>10^{-7}$ mol/l), and not observed during systemic AngII infusion (where the tubular AngII concentration only reaches the nanomoles per liter range).⁴⁵ Interestingly, human proximal tubules studied *in vitro* only exhibit a stimulatory transport response to AngII.⁴⁶

Role of the Proximal Tubule in NHE3 Regulation in Experimental AngII Hypertension

Blood vessels, the kidneys, and the central nervous system are all implicated in the genesis of experimental

hypertension, and T-cells may provide a key link. Animal models of chronic hypertension exhibit increased immune infiltration into the vascular adventitia and kidney.^{47–49} Mice and rats lacking T-lymphocytes exhibit blunted hypertensive responses to experimental hypertension, restored by adoptive transfer of T-cells.^{50,51} Evidence suggests that the initiating insult (whether angiotensin II infusion or other), increases nicotinamide adenine dinucleotide phosphate oxidase-mediated reactive oxygen species generation, which stimulates sympathetic nervous system activity and norepinephrine release in tissues, which can mediate tissue T-cell activation, producing local proinflammatory molecules (eg, reactive oxygen species, neoantigens, and cytokines).^{52,53} In the kidney, these processes are reported to activate local accumulation of AngII, even when systemic levels of AngII are very low.^{54,55} The local AngII is antinatriuretic, produces reactive oxygen species locally, and can attract more immune cells, creating a positive feedback loop that manifests as chronic inflammation and elevated blood pressure.^{49,52} In rats, we determined that AngII-infusion hypertension activates distal transporters and channels, specifically, increasing abundance and phosphorylation of NKCC2 and $\text{Na}^+\text{-Cl}^-$ cotransporters and/or activating proteolytic cleavage of epithelial sodium channels. In contrast, AngII hypertension suppresses proximal tubule and loop of Henle transporters, including NHE3, NaPiII, medullary NKCC2, and medullary Na,K-ATPase.³⁵ In summary, the systemic AngII, inflammation, and intrarenal production of AngII stimulate distal transporters and contribute to hypertension, whereas the resultant hypertension counteracts the effects of the AngII and depresses proximal and loop of Henle sodium transport, facilitating pressure natriuresis.⁵⁶

Many mouse genetic models have been subjected to experimental AngII hypertension and several exhibit a blunted hypertensive response to AngII infusion. We tested the hypothesis that we could identify the locus of the blunting of the hypertension along the nephron: Either reduced AngII stimulation of distal transporters or augmented pressure-natriuretic depression of transporter abundance. This section will focus specifically on the responses of NHE3. In most cases, an augmented suppression of NHE3 abundance accompanied the lower blood pressure, consistent with the notion that local AngII locks NHE3 in the MV and counters the responses to hypertension.

Figure 5 summarizes the measurements of renal NHE3 abundance by immunoblot in wild type (WT) C57Bl/6J mice and genetically modified mice in response to 14-day AngII infusion (490 ng/kg/min). The WT and genetically modified samples were studied

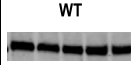
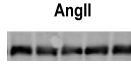
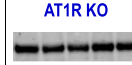
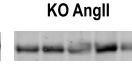
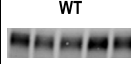
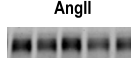
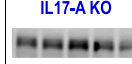
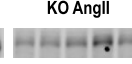
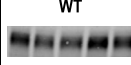
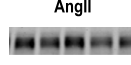
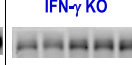
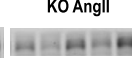
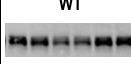
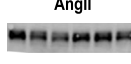
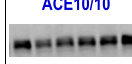
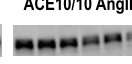
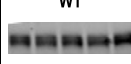
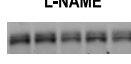
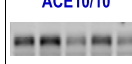
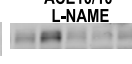
WT	AngII	AT1R KO	KO AngII	citation
				54
1.0 ± 0.07	0.74 ± 0.09	1.0 ± 0.11	0.58 ± 0.07*	
WT	AngII	IL17-A KO	KO AngII	
				55
1.0 ± 0.05	0.95 ± 0.04	1.0 ± 0.07	0.62 ± 0.05*	
WT	AngII	IFN-γ KO	KO AngII	
				55
1.0 ± 0.05	0.95 ± 0.04	1.0 ± 0.05	0.75 ± 0.09*	
WT	AngII	ACE10/10	ACE10/10 AngII	
				57
1.0 ± 0.09	0.99 ± 0.06	1.0 ± 0.14	0.81 ± 0.11	
WT	L-NAME	ACE10/10	ACE10/10 L-NAME	
				52
1.0 ± 0.07	0.85 ± 0.04	1.0 ± 0.15	0.47 ± 0.11*	

Figure 5. In mouse genetic models that exhibit a blunted hypertensive response to angiotensin II (AngII) or N ω -nitro-L-arginine methyl ester hydrochloride (L-NAME) experimental hypertension, sodium hydrogen exchanger 3 (NHE3) abundance is significantly depressed. Models treated for 14 days with AngII infusion by osmotic minipumps include proximal tubule AT1R knockout (KO),⁵⁷ mice with whole body KO of interleukin-17A,⁵⁸ mice with whole body knockout of interferon- γ ,⁵⁸ and in mice with no kidney angiotensin converting enzyme (ACE 10/10).⁶⁰ Immunoblots of a constant amount of renal homogenate are shown for each set of wild-type (WT) and KO mice infused with or without AngII. The last set shows the ACE 10/10 mice infused with L-NAME.⁵⁵ These common findings in disparate models implicate a decrease in NHE3 abundance in the improved pressure natriuresis and blunted hypertension. Additionally, the findings suggest that elevated AngII, cytokines, and reactive oxygen species prevent the redistribution and/or decreased abundance of NHE3 in response to chronic sodium transport stimulation.

at the same time, and results are normalized to mean baseline results defined as 1.0. In the analyses of different sets of WT mice, we did not observe the significant suppression of NHE3 abundance that we measured in rats,³⁵ nor did we detect evidence for AngII stimulation of NHE3; thus, the counteracting influences of hypertension and AngII may balance at the baseline levels in these WT mice. We have not yet analyzed the NHE3 distribution in the MV, nor have we determined whether NHE3 is stimulated during short-term AngII infusion. In mice with a specific genetic deletion of AT1R from the renal proximal tubule (AT1R genetically modified mice), generated by Gurley *et al.*,⁵⁷ the baseline levels of NHE3 were unaltered, yet during AngII infusion NHE3 abundance was decreased 40%, associated with a 20-mm Hg lower blood pressure. In collaboration with the Harrison and Madhur groups⁵⁸ we analyzed mice lacking the ability to synthesize the cytokine interleukin-17A or the cytokine interferon- γ during AngII hypertension. In both genetically modified mouse strains, AngII decreased

abundance of NHE3 (25%–40%), myosin VI (25%), and NaPiII (50%) associated with 20- to 25-mm Hg lower blood pressure and improved natriuretic response to saline infusion. A subsequent study led by the Madhur lab⁵⁹ demonstrated that the interleukin-17A strain had little or no increase in urinary albumin or angiotensinogen during AngII infusion, suggesting that interleukin-17A may reduce intrarenal AngII. Interestingly, the study also demonstrated that interleukin-17A stimulated NHE3 expression in cultured kidney cells mediated by serum and glucocorticoid regulated kinase 1 phosphorylation.

With Gonzalez-Villalobos *et al.*⁶⁰ we investigated the sodium transporter responses in mice that were engineered to express normal systemic ACE but no kidney ACE (ACE 10/10 mice). During AngII infusion, these mice filter and sense-infused AngII, but this AngII nor accompanying inflammatory cytokines cannot stimulate additional local intrarenal production of AngII. In AngII-infused ACE 10/10 mice blood pressure was about 20-mm Hg lower, yet this was not accompanied by a fall in NHE3 abundance. Rather, there was suppression of AngII activation of distal NKCC2 and Na⁺-Cl⁻ cotransporters (NCC).⁶⁰ This same strain was also subject to another distinct model of experimental hypertension caused by inhibition of nitric oxide synthase with the inhibitor N ω -nitro-L-arginine methyl ester hydrochloride (L-NAME). In WT mice L-NAME raises blood pressure 25 mm Hg; suppresses systemic AngII; and through local inflammation, stimulates intrarenal production of AngII. In the ACE 10/10 mice L-NAME did not raise blood pressure, but did significantly suppress NHE3 and sodium phosphate abundance by 50% and 30%, respectively.⁵⁵ A third study in the ACE 10/10 mice demonstrated that this strain is resistant to salt-sensitive hypertension: After washout of the L-NAME treatment, blood pressure returned to normal in both genotypes, yet transporters remained lower in abundance in the ACE 10/10 mice. When both genotypes were subsequently fed a high-salt diet, the WT mice developed a salt-sensitive rise of 20 mm Hg in blood pressure, whereas the ACE 10/10 mice maintained baseline blood pressure.²¹ This differential response can be attributed to the higher inflammation and local production of AngII in the WT mice, and lower NHE3 in the ACE 10/10 mice. Taken together, the findings in these hypertension-resistant mouse models suggest that elevated AngII, cytokines, and/or reactive oxygen species maintain the NHE3 in the MV and blunt the redistribution or decreased abundance of NHE3 in response to sodium transport stimulation along the nephron. In other words, in both AngII and L-NAME hypertension, elevated local AngII production put the brakes on pressure natriuretic adjustments

by activating transporters; thus, a further increase in pressure, and perhaps a different signaling path, is required to decrease transporters.

Summary and Future Directions

Figure 6 provides a simplified overview of the connections among sodium transport stimulation by AngII; cytokines; reactive oxygen species; another important activator, renal sympathetic nerve stimulation; rise in effective circulating volume and blood pressure; and suppression of proximal and loop sodium transporters during hypertension. The results presented in this report suggest that, ultimately, the magnitude of hypertension is determined by the strength of the blood pressure signal(s) required to reduce proximal nephron sodium reabsorption enough to maintain effective circulating volume near baseline. Our conclusion is not intended to ignore the importance of the neuro and vascular aspects of hypertension, but to focus on renal sodium handling. Based on these findings, it is worth considering strategies to facilitate proximal tubule natriuresis as an approach to counteract the stimulatory influences of local AngII. Three candidate pathways appear promising.

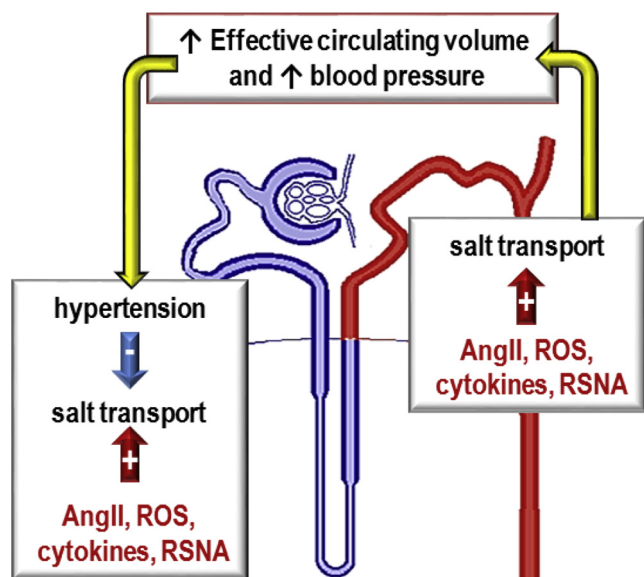


Figure 6. Simplified overview of the connections between sodium transport stimulation by angiotensin II (AngII); cytokines; reactive oxygen species (ROS); and another important activator, renal sympathetic nerve stimulation (RSNA); rise in effective circulating volume and blood pressure; and suppression of proximal and loop sodium transporters during hypertension. The local activation of proximal tubule NHE3 by intrarenal AngII, cytokines, ROS, or RSNA can blunt pressure natriuresis responses and raise blood pressure. Ultimately, the magnitude of hypertension is determined by the strength of the blood pressure signal required to reduce proximal nephron sodium reabsorption (by sodium hydrogen exchanger 3 redistribution to the base of the microvilli or depressed abundance) to restore effective circulating volume. The signals connecting hypertension to antinatriuresis are discussed in the text.

AT2R-Mediated Natriuresis

AT1R and AT2R share similar affinity for AngII, yet AT2R stimulation counteracts the effects of AT1R by increasing bradykinin and nitric oxide release, reducing inflammation, promoting vasodilation and natriuresis.^{61,62} AngIII is the likely ligand for AT2R.⁶³ Hilliard *et al.*^{62,64} showed that direct stimulation of AT2R with the selective agonist C21 increases natriuresis and diuresis without changes in GFR or RBF, evidence for tubular actions. The Carey group confirmed these findings in volume expanded female rats, also showing C21 may move NHE3 to the base of the MV, and that the natriuresis was dependent on nitric oxide and bradykinin.⁶⁵ Hilliard *et al.*⁶⁴ demonstrated that the lower blood pressure and more sensitive pressure natriuresis observed in WT female rodents is associated with 4-fold higher AT2R mRNA; and that this sex advantage disappears with age and in global AT2R KO mice.⁶⁶ This fast-growing area full of therapeutic potential^{61,62} has a large gap in knowledge about how AT2R signaling affects sodium transporters/channels and intrarenal renin angiotensin system in male and female humans.

Glucagon-Like Peptide-1-Mediated Natriuresis

Glucagon like peptide-1 (GLP-1) is an incretin hormone constantly secreted from the intestine at low basal levels in the fasted state. Plasma concentrations rise rapidly after nutrient ingestion. Upon release, GLP-1 exerts insulinotropic effects via a G protein-coupled receptor, stimulation of adenylyl cyclase, and cyclic adenosine monophosphate generation. Although primarily involved in glucose homeostasis, GLP-1 can induce diuresis and natriuresis when administered in pharmacologic doses in humans and rodents. Carraro-Lacroix *et al.*⁶⁷ and Crajoiinas *et al.*⁶⁸ defined the chronic effects of stimulation of the incretin receptor GLP-1 in kidney and discovered that GLP-1 has diuretic and natriuretic effects mediated by changes in both renal hemodynamic parameters and by down-regulation of proximal tubule NHE3 activity.⁶⁸ Recently, Farah *et al.*⁶⁹ demonstrated that endogenous baseline GLP-1 plays a significant role in regulating renal function. They blocked the GLP-1 receptor with the antagonist exendin-9 in overnight-fasted anesthetized rats. Exendin-9-infused (30 minutes) rats exhibited reduced GFR, lithium clearance, urinary volume flow, and sodium excretion compared with vehicle-infused controls. NHE3 phosphorylation at a site associated with retraction to the base was also increased. Collectively, these results provide novel evidence that GLP-1 is a physiologically relevant natriuretic factor that contributes to sodium balance, in part, via tonic modulation of sodium transport activity in the proximal tubule.

Sodium-Glucose-Cotransporter 2 Inhibitors

The sodium-glucose-cotransporter inhibitors used to treat diabetes directly target the proximal tubule, provoke natriuresis and diuresis, and may lower blood pressure.⁷⁰ Pessoa *et al.*⁷¹ recently reported that NHE3 activity is stimulated by luminal glucose, and that NHE3 colocalizes and may functionally interact with sodium-glucose-cotransporter 2 in the proximal tubule. Thus, it is possible that sodium-glucose-cotransporter 2 inhibition may inhibit NHE3 transport activity.

Dopamine Receptor-Mediated Natriuresis

The intrarenal dopaminergic system is an important determinant of the blood pressure set point: Reduced dopamine signaling is associated with hypertension.^{72–74} There are 5 dopamine receptors in the kidney: D1R and D5R (D1likeR) physically interact and their activation inhibits NHE3 and Na,K-ATPase⁷⁵; thus, counteracting the effects of AngII via AT1R.⁶³ D1likeR activation increases salt and water excretion mediated by inhibition of proximal tubule NHE3, sodium phosphate, and Na,K-ATPase as well as loop of Henle NKCC2, the proximal sites that can elicit pressure natriuresis.⁷⁶ Additionally, dopamine receptor activation is known to antagonize AT1R signaling, reduce intrarenal renin angiotensin system components,^{63,76,77} and stimulate AT2R signaling.⁷⁸ Numerous labs have provided evidence for functional complexes between Ang AT1R and DA D1R⁷⁹ in which activation of one attenuates the expression of the other. Likewise, AT2Rs may oppose AT1Rs by protein–protein interaction.⁶³ Thus, effects of dopamine deficiency include AT1R activation and vice versa.

Determining how the pressor and natriuretic arms of the renin angiotensin system, in parallel with the interacting dopaminergic system, regulate transporters and channels may fill important gaps in understanding the sexual dimorphism of blood pressure and provide new and sex-specific therapeutic approaches to treat resistant hypertension.

DISCLOSURE

The author declared no competing interests.

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