Hypertension is the leading cause of stroke and cardiovascular diseases and a leading risk factor for global disease burden, affecting 30% of the adult population in Western cultures. Blood pressure (BP) can be elevated by vasoconstriction and by increasing the circulating volume. Evidence from studies of mutations in renal Na⁺ transporters, renal transplantation, and diuretic action supports Guyton’s hypothesis that long-term regulation of effective circulating volume and BP depends on fractional renal Na⁺ reabsorption. Ultimately, excess Na⁺ reabsorption raises effective circulating volume and BP which provoke counteracting natriuretic responses to match Na⁺ output to Na⁺ intake at the expense of circulating volume. We and others have determined that pressure natriuresis responses involve Na⁺ transporters. According to Guyton, kidneys possess the capacity to excrete enough Na⁺ and volume to normalize BP in the face of expanded effective circulating volume. Thus, hypertension can be characterized as a failure of compensatory renal pressure natriuresis. Indeed, there is strong evidence that the pressure natriuresis response is impaired during experimental hypertension by inflammation, immune cell infiltration, and intrarenal angiotensin II (AngII) production, secondary to initiating stimuli such as AngII infusion, reduced NO production, high-salt diet, or elevated renal sympathetic nerve activity. This brief review focuses on the natriuretic effectors and addresses (1) the renal tubular locations and transporters that participate in pressure natriuresis and (2) the mechanisms that blunt the response in experimental models of hypertension.

Effectors of Pressure Natriuresis

Pressure natriuresis describes the responses to acute or chronic increases in BP. Chou and Marsh, using video microscopy, provided compelling evidence that the proximal tubule was a site of pressure natriuresis: raising BP by acutely constricting the vasculature rapidly increased the flow leaving the proximal tubule by 50%. They concluded that the depressed proximal tubule reabsorption increased fluid load signal at the macula densa, contributing to the glomerular filtration rate and renal blood flow autoregulation evident during increased renal perfusion pressure, and that the measured increased distal delivery of fluid and salt could account for the magnitude of the pressure natriuresis and diuresis. Proximal tubule reabsorbs two-thirds of the filtered Na⁺ and volume at baseline and this fraction is decreased not only when BP is increased but also during a high-salt diet (facilitated by local dopamine production) and when the renin angiotensin system is inhibited. Working with the Marsh group, we discovered that these variables all regulate the distribution of the proximal tubule sodium–hydrogen exchanger isoform 3 (NHE3) and the sodium–phosphate cotransporter isoform 2 (NaPi2) between the top and the base of the apical microvilli of the proximal tubule. Natriuretic stimuli provoke the dynamic redistribution of these transporters, along with associated regulators, molecular motors (myosin VI, IIA), and cytoskeleton-associated proteins, to the base of the microvilli. During acute hypertension, the lipid raft–associated NHE3 remains at the base, and the non–raft-associated NaPi2 is endocytosed, culminating in decreased Na⁺ transport activity measured as increased proximal tubule flow rate. Further along the nephron, an analysis of the response of the distal convoluted tubule to acute hypertension revealed that the sodium chloride cotransporter (NCC) retracted from apical membranes to subapical cytoplasmic vesicles, providing evidence for its participation in the pressure natriuresis. Importantly, the NCC redistribution was driven by the fall in AngII that accompanies acute hypertension rather than hypertension per se.

In contrast to pressure natriuresis, antinatriuretic stimuli (AngII, renal sympathetic nerve activity, and low-salt diet) redistribute the same proximal tubule transporters into the body of the microvilli associated with an increase in transport activity measured as decreased proximal tubule flow rate. Along the distal nephron, acute AngII stimulation provokes redistribution of NCC into the apical plasma membrane. Although abundance of the phosphorylated NCC was not increased by acute AngII treatment, this phosphorylated NCC clustered within the apical membrane as multimeric complexes, creating regions with elevated phosphorylated NCC to NCC total ratio (assessed by subcellular fractionation and blue native gels). As reviewed below, antinatriuretic stimuli increase and oppose pressure natriuresis during chronic hypertension, necessitating a further increase in BP, the error signal driving the response, to recruit additional antinatriuretic mechanisms to balance Na⁺ output to Na⁺ intake.
Chronic AngII hypertension, one of the well-studied models of experimental hypertension, involves the controlled infusion of a subpressor dose of AngII for a couple of weeks. Because transporter regulation in one region of the nephron can drive transporter regulation in other regions, we analyzed renal transporter regulation along the entire nephron, building on the transporter profiling approach developed by the Knepper group. This approach, implemented in homogenates of renal cortex and medulla, is facilitated by the anatomic arrangement of sodium transporters and channels along the nephron (Figure A) and the availability of specific antibodies: renal cortical NHE3 and NaPi2 are localized to the proximal tubule, medullary NHE3 and sodium–potassium–2 chloride cotransporter (NKCC2) to the medullary thick ascending limb of the loop of Henle. Sodium–potassium-2 chloride cotransporter (NKCC2; target of loop diuretics) is expressed in both medulla and cortex along the thick ascending limb. Sodium chloride cotransporter (NCC; target of thiazide diuretics) is localized to the cortical distal convoluted tubule and SPAK (kinase that activates NKCC2 and NCC) is expressed in both cortex and medulla from thick ascending limb through distal convoluted tubules. ENaC (target of potassium sparing diuretics) α, β, and γ subunits are expressed in the cortex from late distal convoluted tubule through to principal cells of the cortical collecting duct, as well as in the medullary collecting ducts.

We tested, in male rats, the hypothesis that AngII infusion (400 ng/kg per minute for 14 days) would activate Na⁺ transporters in the distal nephron, which would drive compensatory inhibition of proximal tubule transporters to maintain Na⁺ and volume homeostasis. Cardiac hypertrophy and increased urinary sodium excretion were consistent with a pressure natriuresis response. The results, summarized in Figure (B), demonstrate that AngII infusion increased the abundance and activating phosphorylation of cortical transporters including NKCC2, NCC, and their phosphorylated forms all increase significantly (NCCpT53 and NCCpS89 also significantly increase 5- and 3-fold, respectively), cortical α-ENaC and β-ENaC as well as cleaved α-ENaC and γ-ENαC subunits significantly increase. In contrast, cortical NHE3 and medullary thick limb NHE3, NKC, and sodium pump α and β subunits (α-Na,K-ATPase [NKA], β-NKA), SPAK and SPAKp are all significantly depressed in abundance during AngII hypertension.

Figure. Renal transporter profile reveals region-specific transporter regulation during experimental hypertension. A, Anatomic arrangement of renal cortex and renal medulla in a kidney cross-section with adjacent drawing of a single nephron indicating locations of sodium transporters, epithelial sodium channel (ENaC), and Ste20/SPS-1-related proline-alanine kinase (SPAK). Cortical sodium–hydrogen exchanger isoform 3 (NHE3) and sodium–phosphate cotransporter isoform 2 (NaPi2) are primarily restricted to the proximal tubules where two-thirds of the filtered load is reabsorbed. Medullary NHE3 is expressed in the S3 portion of the proximal tubule that terminates in the medulla as well as in the thick ascending limb of the loop of Henle. Sodium–potassium-2 chloride cotransporter (NKCC2; target of loop diuretics) is expressed in both medulla and cortex along the thick ascending limb. Sodium chloride cotransporter (NCC; target of thiazide diuretics) is localized to the cortical distal convoluted tubule and SPAK (kinase that activates NKCC2 and NCC) is expressed in both cortex and medulla from thick ascending limb through distal convoluted tubules. ENaC (target of potassium sparing diuretics) α, β, and γ subunits are expressed in the cortex from late distal convoluted tubule through to principal cells of the cortical collecting duct, as well as in the medullary collecting ducts. B, Effects of angiotensin II (AngII) infusion into rats (400 ng/kg per minute for 14 days) replotted from Nguyen et al expressed as protein abundance relative to mean abundance in control untreated rats, defined as 1.0. In summary, distal nephron NKCC, NCC, SPAK, and their phosphorylated forms all increase significantly (NCCpT53 and NCCpS89 also significantly increase 5- and 3-fold, respectively), cortical α-ENaC and β-ENaC as well as cleaved α-ENaC and γ-ENαC subunits significantly increase. In contrast, cortical NHE3 and medullary thick limb NHE3, NKC, and sodium pump α and β subunits (α-Na,K-ATPase [NKA], β-NKA), SPAK and SPAKp are all significantly depressed in abundance during AngII hypertension. C, Integration of responses to maintain fluid balance: antinatriuretic stimuli including AngII, reactive oxygen species (ROS), cytokines, and renal sympathetic nervous system activity (RSNA) can stimulate salt reabsorption (red arrows) increasing effective circulating volume and blood pressure which can suppress salt transporters (blue arrow). Cleaved indicates ENaC subunits cleaved to smaller molecular weight forms associated with channel activation; FL, full length form; and p, phosphorylated form.
limb as another locus of pressure natriuresis. Consistent with this idea, medullary NKCC2 and SPAK abundance were also decreased 40% to 50%, in contrast to their activation in the cortex during AngII hypertension. Medullary sodium pump Na,K-ATPase subunits, driving sodium reabsorption, were likewise decreased significantly during AngII infusion. The concerted decreases in abundance of NHE3, NKCC2, and Na,K-ATPase (Figure [B]) support the conclusion that the thick ascending limb participates in pressure natriuresis along with the proximal tubule (Figure [C], blue nephron region). This conclusion was already evident from the Cowley laboratory findings demonstrating a key role of medullary thick ascending limb in driving normal pressure natriuresis, specifically, the elevated medullary blood flow driven by renal interstitial hydrostatic pressure, as well as the medullary redox state influenced by NO production, 2 responses blunted in chronic hypertension.

These results revealed that AngII hypertension increases transporters' abundance and activation from the cortical thick limb to the medullary collecting duct (NKCC2, NCC, ENaC, and regulatory kinase SPAK) and that this stimulation is balanced by a compensatory inhibition of transporters from proximal tubule through medullary thick limb (cortical NHE3 and medullary: NHE3, NKCC2, Na,K-ATPase, SPAK), presumably driven by elevated BP (Figure [B and C]). Can proximal suppression be attributed to the inhibitory actions of AngII reported at concentrations above $10^{-7}$ mol/L? Direct measures of proximal tubule fluid (AngII) indicated concentrations 10-fold higher than in plasma at baseline, and further elevated by AngII infusion because of local AngII production, yet concentrations were in the nanomolar range. 100X less than the doses reported to inhibit proximal tubular reabsorption. The authors concluded that the elevated proximal tubule (AngII) impairs pressure natriuresis.

This analysis of sodium transporter regulation along the nephron during AngII hypertension in rat defined how the effective circulating volume is maintained during the opposing forces of AngII and hypertension and revealed region- and context-specific regulation of NKCC2 and SPAK in medulla versus cortex.

**Novel Arginine Methyl Ester Hypertension**

NO is a vasodilator and natriuretic that inhibits NHE3, NKCC, and ENaC in vitro. L-NAME (Novo-L-arginine methyl ester) inhibits NO synthase, lowers NO levels, and raises BP. With Gonzalez-Villalobos and colleagues, we examined the transporter profile in kidneys from mice with L-NAME hypertension. In addition to low NO, this model exhibits low circulating AngII, elevated intrarenal AngII production, and vasoconstriction. Thus, one might expect renal transporters' stimulation in the absence of the NO; however, no distal transporter activation was evident. Rather, NHE3, NKCC2, ENaC subunits, and the regulatory kinase SPAK were all suppressed; NCC was unaltered. L-NAME was previously reported to depress these sodium transporters in a model incorporating aldosterone plus high-salt diet. These decreases theoretically facilitate sodium and volume excretion during persistent vasoconstriction and illustrate that the pressure natriuresis mediators can extend to the collecting duct if needed to match sodium excretion to sodium intake.

**Genetic Models With Resistance to AngII Hypertension**

Many genetic mouse models exhibit blunted hypertensive responses to AngII infusion compared with the response in wild-type mice. We reasoned that a transporter profile approach could be applied to determine whether the blunting was because of less transporter activation by AngII or because of more effective pressure natriuresis. The results of 3 genetic models will be discussed. One caveat is that these studies were carried out in whole kidneys before we characterized differential regulation along the thick limb of the loop of Henle in the rat model.

**Proximal Tubule–Specific Knockout of the Angiotensin Type 1 Receptor**

Gurley and Coffman created a mouse that did not express the angiotensin type 1 receptor (AT1R) in the proximal tubule (AT1R PTKO) and found that, in response to 2-week AngII infusion, BP increased 10 mmHg less than wild-type controls. Although NHE3 and NKCC2 were depressed 25% and 30%, respectively, in AngII-infused wild-type mice, NHE3 was reduced further to 50% of baseline, and proximal NaPi2 was reduced 40% in AngII-infused AT1R PTKO. Supporting improved pressure natriuresis in the AT1R PTKO, Schnermann demonstrated by micropuncture that proximal tubule fractional absorption was reduced from 44% to 36%. AngII activation of proximal tubule AT1R is also implicated in the stimulation of local synthesis and accumulation of AngII, a pathway that is likely suppressed in this model.

**Mice With No Kidney**

Angiotensin-Converting Enzyme

Gonzalez-Villalobos and Bernstein analyzed the responses to AngII hypertension in mice that were engineered to express angiotensin-converting enzyme (ACE) only in myelomonocytic cells. These mice (termed ACE 10/10) have normal BP and negligible amounts of intrarenal ACE, thus, cannot produce intrarenal ACE during experimental hypertension. During chronic AngII infusion, BP rose 20 mmHg less in ACE 10/10 than wild-type mice indicating that intrarenal ACE contributes to the hypertension. Transporter profiling of wild-type and ACE 10/10 mice infused with AngII revealed that the activation of distal transporters and regulatory kinases (including NKCC2, NCC, pendrin, and SPAK) evident in the wild type was effectively prevented in the ACE 10/10 mice supporting the idea that this activation was dependent on intrarenal AngII production. The physiological significance of the transporter changes during AngII was demonstrated using diuretic tests that showed increased thiazide and furosemide-sensitive Na+ excretion in wild-type but not in ACE 10/10. Interestingly, ENaC subunits' activation by cleavage was similar in both wild-type and ACE 10/10 during AngII infusion, suggesting that the remaining BP elevation is because ENaC is persistently activated by the infused AngII or aldosterone stimulation. Unlike the results in the rat and AT1R PTKO studies, NHE3 abundance did not significantly decrease in...
wild-type or ACE10/10 mice in response to AngII in this study. Perhaps, this is because of the lower amount of AngII infused and lower BP attained in the study by Gonzalez-Villalobos et al. (400 ng/kg per minute and 150 mm Hg) compared with the study by Gurley et al. (1000 ng/kg per minute and 170 mm Hg). Regardless, the NHE3 abundance was not increased by AngII infusion. The dependence of NHE3 abundance regulation on the degree of hypertension versus the amount of AngII remains to be explored.

The effects of chronic L-NAME treatment were also examined in the ACE 10/10 mice. Although BP increased to ∼140 mm Hg in the wild-type mice treated with L-NAME, ACE 10/10 mice exhibited a strong natriuretic response to the NO synthase inhibitor and were completely protected from L-NAME hypertension. L-NAME provoked larger decreases in transporters’ abundance and phosphorylation all along the nephron in the ACE 10/10 (≥50% decreases in NHE3, phosphorylated NKCC, phosphorylated NCC, and SPAK) compared with those observed in the wild types. Thus, the full potential of the pressure natriuresis response in L-NAME hypertension becomes evident in the absence of local AngII production. In both AngII and L-NAME hypertension, local production of AngII activates sodium transporters which puts the brakes on pressure natriuretic adjustments, thus BP must increase further to activate natriuretic mediators that suppress transporter activity.16,34

Mice Lacking Cytokine Production

Harrison and colleagues demonstrated that mice lacking T lymphocytes present blunted hypertensive responses to experimental hypertension, restored by adoptive transfer of T cells. The same group recently provided evidence that a population of CD8+ T cells infiltrate the kidneys during AngII infusion and produce the cytokines interferon-γ (IFN-γ) and interleukin-17 (IL-17). In collaboration with the Harrison group, we investigated the roles of these specific cytokines by profiling transporters in IFN-γ knockout (IFN-γ−/−) and IL-17A knockout mice (IL-17A−/−) after 2-week AngII (490 ng/kg per minute). Systolic pressure increased >40 mm Hg in wild-type mice and <20 mm Hg in IFN-γ−/− and IL-17A−/− mice. In addition, natriuretic responses to a saline volume expansion were suppressed in wild types, but not in IL-17A−/− and IFN-γ−/− during AngII infusion. Despite similar blunting of hypertension in both IL-17A−/− and IFN-γ−/−, the transporter profiles during AngII infusion were distinct. Activation (increased phosphorylation) of distal NKCC2, NCC, and SPAK, evident in the wild-type mice, was preserved in IL-17A−/− but prevented in the IFN-γ−/− genotype; ENaC activation persisted in all 3 genotypes. In both IL-17A−/− and IFN-γ−/−, proximal NHE3, NaPi2, and the motor myosin VI were significantly depressed during AngII infusion (25%–50%). Taken together, the similar blunting of hypertension in the 2 distinct cytokine knockout genotypes can be attributed to the suppression of proximal NHE3, NaPi2, and myosin VI evident in both genotypes, and the role of the distal activation of NKCC, NCC, and SPAK during AngII infusion, evident in IL-17A−/− but not in IFN-γ−/−, remains an open issue. As in the ACE 10/10 genotype, the remaining hypertension in the IL-17A−/− and IFN-γ−/− may be attributed to the persistent ENaC activation.

In conclusion, transporter profiling can pinpoint where transporters and channels are activated and suppressed along the nephron during hypertension. Although not the focus of this review, distal anion transporters and potassium channels can also affect the BP set point and warrant inclusion in future comprehensive profiling. The profiles of the 4 genotypes illustrate improved pressure natriuresis potential during experimental hypertension and shed some light on how this potential is blunted in wild-type mice, that is, infused and locally produced AngII and cytokines put the brakes on natriuresis in response to elevated effective circulating volume and hypertension (Figure C). Eliminating proximal tubule AT1R, intrarenal ACE, or whole body IFN-γ or IL-17A releases the brakes, at least in part, and improves natriuretic potential. Understanding the molecular mechanisms connecting these antinatriuretic mediators to the transporter activation has the potential to lead to strategies to amplifying the pressure natriuretic responses of the proximal tubule and thick ascending limb and, theoretically, reduce the BP set point needed to maintain effective circulating volume homeostasis.

Mediators of Pressure Natriuresis

This exploration of transporter regulation during hypertension brings us to the question, “What are the signals driving the transporter changes that cause pressure natriuresis?” Multiple signals have the potential to suppress sodium reabsorption and we assume the pressure natriuresis response is the sum of the prevailing natriuretic and antinatriuretic influences along the nephron. Previous studies established that acutely raising BP stimulates nonautoregulating preglomerular vascular elements to release mediator(s) that can inhibit sodium transport in nearby tubules. Inhibiting cytochrome P-450 metabolism blunts the diuretic response to acute hypertension and prevents redistribution of NHE3 and inhibition of Na,K-ATPase. The Carey laboratory provided evidence that an increase in renal interstitial cGMP is important to drive the natriuresis, supporting a role for intrarenal NO. Interestingly, this response would be lacking in L-NAME hypertension, where transporters’ abundance is nevertheless suppressed, presumably via a different path.

Clamping AngII at a suppressor levels during acute hypertension blunts pressure natriuresis ≥50% and prevents retraction of both NHE3 from the proximal tubule microvilli and NCC from apical membranes of the distal convoluted tubule. These findings are relevant to the blunting of pressure natriuresis in chronic AngII or L-NAME hypertension in which intrarenal AngII levels are elevated. Activation of the local production of dopamine in the proximal tubule warrants consideration as a pressure natriuretic mediator as it inhibits NHE3 and Na,K-ATPase activity, dopamine receptor inactivation raises BP, and reductions in activity of either the local renin angiotensin system or the dopamine system increase the activity of the other. Thus, suppression of natriuretic
dopamine is predicted if local production of AngII is amplified. Another important factor to consider is renal sympathetic nervous system activation secondary to renal injury which prevents retraction of NHE3 out of the microvilli in the face of hypertension and blunts natriuresis.5,55,56

We propose, for further investigation, that the initial natriuretic response to acute hypertension is driven by rapid generation of mediators (20-HETE, NO, perhaps dopamine) released locally and that subsequent renin angiotensin system and renal sympathetic nerve activity inhibition are important to sustain the natriuresis during chronic hypertension by reducing sodium transport along the nephron (as illustrated in L-NAME hypertension).16,34 The pressure natriuresis response can be impaired during chronic hypertension by inflammation, immune cell infiltration, intrarenal AngII production, reduced NO production, high-salt diet, and elevated renal sympathetic nerve activity provoking chronic hypertension (Figure [C]).14–17

Perspectives and Future Directions
This critical blood pressure setting response is understudied relative to its therapeutic potential. The studies summarized in this brief review focused on sodium transport regulation in the face of simultaneous AngII stimulation and the opposing antinatriuretic signal of hypertension and demonstrate the rationale and necessity for addressing transporter regulation along the entire nephron using whole animal models. Although we can assess transporters’ abundance, covalent modifications, and subcellular distribution, fewer tools are available to investigate the impact of transporter changes beside lithium clearance, saline challenges, and diuretic tests. An exception is the study, discussed above, visualizing hypertension-stimulated redistribution of NHE3 to the base of the microvilli using in vivo microscopy.57 Further innovative efforts to examine in vivo responses to stimuli such as hypertension and renal injury, especially during a longer time courses, would undoubtedly answer open questions about how pressure natriuresis interfaces with antinatriuretic stimuli and intrarenal controls, including autoregulation, to produce a natriuresis that equals sodium intake. At the level of transporter regulation, we have focused on abundance and phosphorylation in this brief review, but we and others have also provided evidence for importance of membrane lipid domain localization, ubiquitination, and protein–protein interactions. Other potential transporter modifications warrant investigation in vivo models. About the integration of activating and inhibitory signaling pathways during hypertension, genetic models have proven invaluable and have generated even more questions for study, for example, the key role of immune cell infiltration. Future studies will likely benefit from tubule-specific and inducible genetic modifications. In conclusion, although the field (my laboratory included) has focused on how and why sodium transport is aberrantly elevated in specific tubular regions during hypertension, we provide a rationale for broadening investigations to the entire nephron to obtain an integrated understanding of how the kidney generates a natriuresis to maintain fluid and electrolyte balance in the face of effective circulating volume and BP elevation.

Sodium Transporter Profiling During Hypertension

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References


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