Cell Metabolism
Short Article

AT_{1A} Angiotensin Receptors in the Renal Proximal Tubule Regulate Blood Pressure

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SUMMARY

Hypertension affects more than 1.5 billion people worldwide but the precise cause of elevated blood pressure (BP) cannot be determined in most affected individuals. Nonetheless, blockade of the renin-angiotensin system (RAS) lowers BP in the majority of patients with hypertension. Despite its apparent role in hypertension pathogenesis, the key cellular targets of the RAS that control BP have not been clearly identified. Here we demonstrate that RAS actions in the epithelium of the proximal tubule have a critical and nonredundant role in determining the level of BP. Abrogation of AT_1 angiotensin receptor signaling in the proximal tubule alone is sufficient to lower BP, despite intact vascular responses. Elimination of this pathway reduces proximal fluid reabsorption and alters expression of key sodium transporters, modifying pressure-natriuresis and providing substantial protection against hypertension. Thus, effectively targeting epithelial functions of the proximal tubule of the kidney should be a useful therapeutic strategy in hypertension.

INTRODUCTION

Among the regulatory systems for blood pressure (BP), the RAS has a dominant role (Le et al., 2008). Pathological activation of the RAS is a common contributor to hypertension in humans as RAS antagonists lower BP in the majority of patients with essential hypertension (Matchar et al., 2008). The actions of the RAS to increase BP are primarily mediated by activation of type 1 (AT_1) angiotensin receptors. AT_1 receptors are expressed in a number of tissues where they have a potential to affect BP including the CNS, heart, vasculature, kidney, and adrenal gland (Le et al., 2008), but it has been difficult to identify the critical tissue targets of the RAS in hypertension pathogenesis.

Recent studies have implicated vascular signaling pathways as key contributors to BP regulation and development of hypertension (Guilluy et al., 2010; Heximer et al., 2003; Michael et al., 2008; Wirth et al., 2008). On the other hand, the work of Guyton and colleagues (Guyton, 1991), human genetic studies by the Lifton laboratory (Lifton et al., 2001), and our recent studies in mice (Coffman and Crowley, 2008) have suggested that renal excretory function is a major determinant of intra-arterial pressure. In the kidney, AT_1 receptors are expressed in epithelial cells along the nephron (Bouby et al., 1997). Among populations of renal epithelia, AT_1 receptors in the proximal tubule may have special relevance to BP homeostasis because this segment is responsible for reabsorption of a sizeable fraction of the glomerular filtrate (Weinstein, 2008), it contains all of the components of the RAS under independent local control (Kobori et al., 2007; Navar et al., 2002), and RAS activation is known to influence its handling of solutes and fluid (Cogan, 1990). Nonetheless, while direct actions of angiotensin II in the proximal tubule were first identified more than 25 years ago (Schuster et al., 1984), their impact on regulation of BP in the intact animal has never been clearly defined. Here we demonstrate potent actions of AT_1 receptors in renal proximal tubule to regulate BP homeostasis.

RESULTS

Reduced BP in Mice Lacking AT_{1A} Receptors in the Renal Proximal Tubule

We crossed mice with a conditional *Agtr1a* allele (Figure S1, available online) with a *Pepck-Cre* transgenic mouse line expressing *Cre* in proximal but not distal nephron segments (Rankin et al., 2006; Figures S2A and S2B) to generate mice lacking AT_{1A} receptors only in the renal proximal tubule (PTKO). As shown in Figure 1A, systolic BPs measured by radiotelemetry were significantly lower in PTKO mice (126 ± 3 mm Hg) than littermate controls (136 ± 3 mm Hg; $p = 0.03$). This difference was apparent both during the day (120 ± 3 versus 130 ± 3 mm Hg; $p = 0.003$) and at night (133 ± 3 versus 142 ± 3 mm Hg; $p = 0.04$). Mice were then sequentially fed high-salt (6% NaCl) and low-salt (<0.002% NaCl) diets while their BPs were

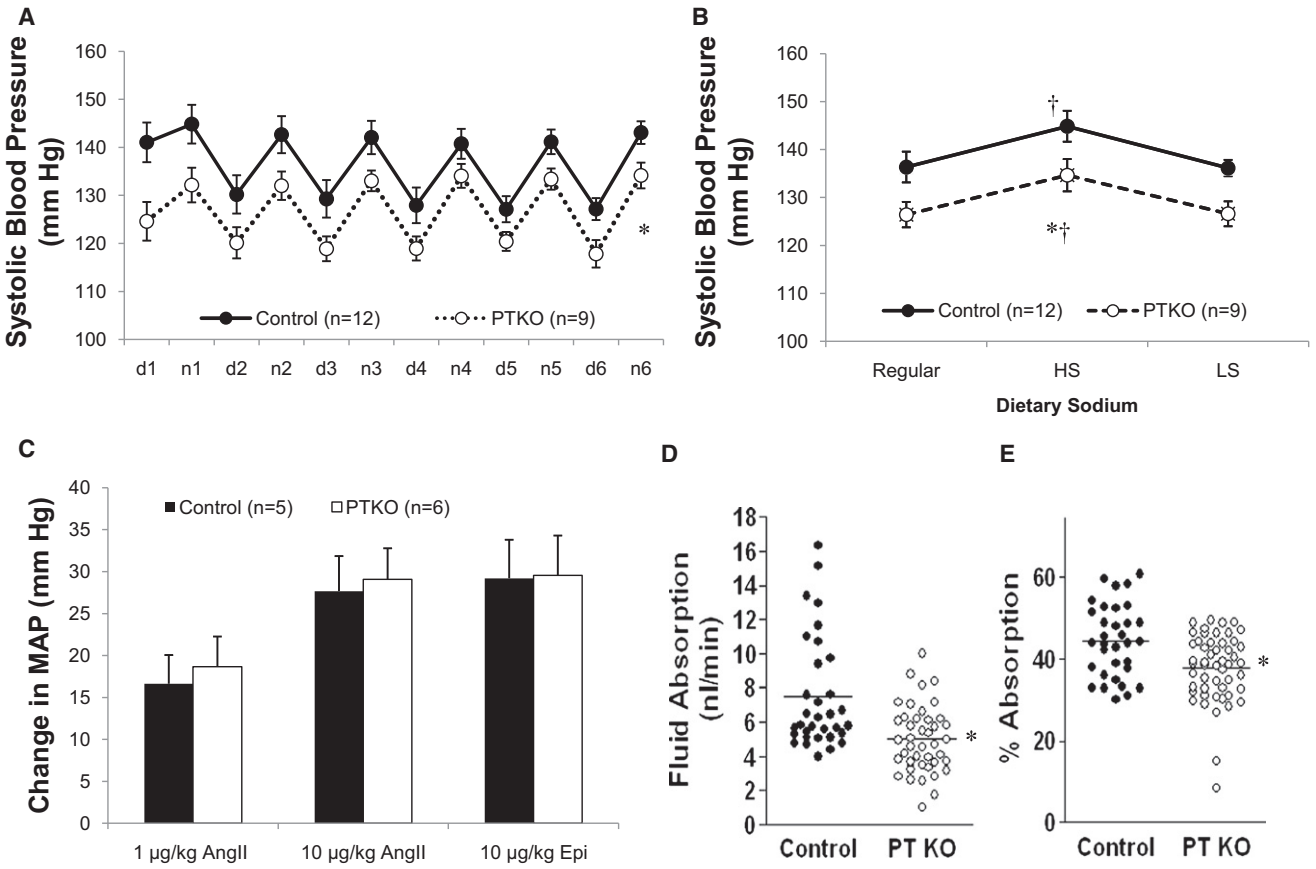

Figure 1. Baseline Studies in PTKO Mice

(A) Twelve hours mean systolic BPs during the day (d) and at night (n) on a control diet. Systolic BPs were significantly lower in PTKO mice compared to controls (*$p = 0.03$).

(B) BPs increased significantly (†$p < 0.01$) and to a similar extent in PTKOs and controls during high-salt (6% NaCl) feeding and returned to baseline values on low-salt (<0.02% NaCl) feeding. BPs were significantly lower in the PTKOs throughout the experiment (*$p < 0.044$).

(C) The maximal increases in mean arterial pressure (MAP) compared to baseline in response to bolus infusions of angiotensin II (AngII, 1 and 10 μg/kg) or epinephrine (Epi, 10 μg/kg) were identical between PTKOs and controls.

(D) Rates of fluid reabsorption by the renal PT measured in vivo by using standard free-flow micropuncture were significantly reduced in the PTKO mice compared to controls as an absolute rate ($p = 0.00011$) or (E) when adjusted for single-nephron glomerular filtration rate ($p = 0.0007$). Error bars represent SEM.

monitored. As shown in Figure 1B, BPs increased significantly and to a similar extent in both groups during high-salt feeding and returned to baseline levels when the low-salt diet was instituted, consistent with the phenotype of sodium sensitivity previously reported in 129 mice (Francois et al., 2005); however, the magnitude of BP difference between the PTKOs and controls remained constant across the different dietary sodium intakes.

To exclude the possibility that vascular responses mediated by AT_1 receptors might be affected in the PTKOs, we assessed acute pressor responses to angiotensin II in vivo as described previously (Ito et al., 1995). As shown in Figure 1C, acute infusion of angiotensin II caused marked vasoconstriction and the magnitude of the vasoconstrictor responses was virtually identical in both groups.

AT_{1A} Receptors in the Proximal Tubule Control Fluid Reabsorption

To determine whether deletion of AT_{1A} receptors would affect fluid handling in the proximal tubule, we examined nephron function by using free-flow micropuncture (Hashimoto et al., 2005). As shown in Table 1 and Figure 1D, absolute rates of proximal fluid reabsorption (4.76 ± 0.32 versus 7.5 ± 0.58 nl/min; $p = 0.00014$) were significantly reduced in the PTKOs compared to controls. Single-nephron GFRs measured in the proximal tubule were also significantly lower in the PTKOs (13 ± 0.61 nl/min) than controls (16.54 ± 0.81 nl/min; $p = 0.001$), consistent with the whole-animal values (Table 1). Nonetheless, when corrected for SNGFR, fractional reabsorption rates in the proximal tubule were also significantly lower in the PTKOs (36.5 ± 1.5%) than controls (44.5 ± 1.6%; $p = 0.0005$, Figure 1E). Thus, loss of AT_{1A} receptors from the proximal tubule leads to a reduction in fluid reabsorption, indicating a tonic role for the RAS to control fluid reabsorption by proximal tubule epithelia in vivo.

PTKO Mice Are Protected Against Hypertension

To determine the contribution of AT_{1A} receptors in the proximal tubule to the development of hypertension, we infused PTKO and control mice with angiotensin II (1000 ng/kg/min) by osmotic

Table 1. Physiological Data

	Control (n = 8)	PT KO (n = 7)	p Value
Body weight (g)	32.4 ± 1.73	30 ± 1.54	ns
Kidney weight (mg)	435.4 ± 19.8	410 ± 22.1	ns
Glomerular filtration rate (GFR, μl/min)	620 ± 49	439 ± 58	0.025
U Osm (mOsm/kg H_2O)	2441.4 ± 103	2165 ± 102	0.15
UV (μl/min)	1.5 ± 0.08	2.1 ± 0.17	0.005
Proximal Tubule Micropuncture			
TF/P iothal	1.85 ± 0.05	1.6 ± 0.03	0.0002
Absorption (nl/min)	7.5 ± 0.58	4.76 ± 0.32	0.000
Fractional abs (%)	44.45 ± 1.57	36.5 ± 1.53	0.0005

Data represent values ± SEM.

minipump. As shown in Figure 2A, the control group developed robust hypertension with a mean systolic BP of 172 ± 4 mm Hg, which was significantly higher than that of the PTKOs (150 ± 4 mm Hg; p = 0.0005). A similar difference was observed comparing the mean increase in BPs in the controls (38 ± 5 mm Hg) with the PTKOs (23 ± 3 mm Hg; p = 0.0005) (Figure 2B). Thus, deletion of AT_{1A} receptors from the proximal tubule conveyed substantial protection against hypertension. As shown in Figure 2C, the extent of positive sodium balance was significantly reduced in PTKOs (0.33 ± 0.04 mmol) compared to controls (0.50 ± 0.07 mmol; p = 0.046), consistent with facilitated natriuresis as a mechanism for their resistance to hypertension.

Control of Renal Sodium Transporters by AT_{1A} Receptors in Proximal Tubule

To examine the molecular mechanisms responsible for the attenuated hypertension and enhanced pressure natriuresis in PTKOs, we measured abundance of key sodium transporters along the nephron (Figure 3). At baseline, there were no differences in abundance of any of these transporters between PTKOs and controls. However, during angiotensin II infusion, significant differences in pool sizes of key transporters were uncovered. In control mice, abundance of the sodium-proton antiporter 3 (NHE3), the major sodium transporter in the proximal tubule, fell by ≈30% during angiotensin II infusion (Figures 3A and 3B). By contrast, in the PTKOs, abundance of NHE3 fell by more than 50% (Figures 3A and 3B) to levels that were significantly lower than controls (0.50 ± 0.06 versus 0.70 ± 0.09; p = 0.01). In control mice, levels of the sodium phosphate cotransporter (NaPi2) were unaffected by angiotensin II infusion (Figures 3C and 3D), whereas NaPi2 protein levels decreased by ≈36% in the PTKOs (p = 0.005).

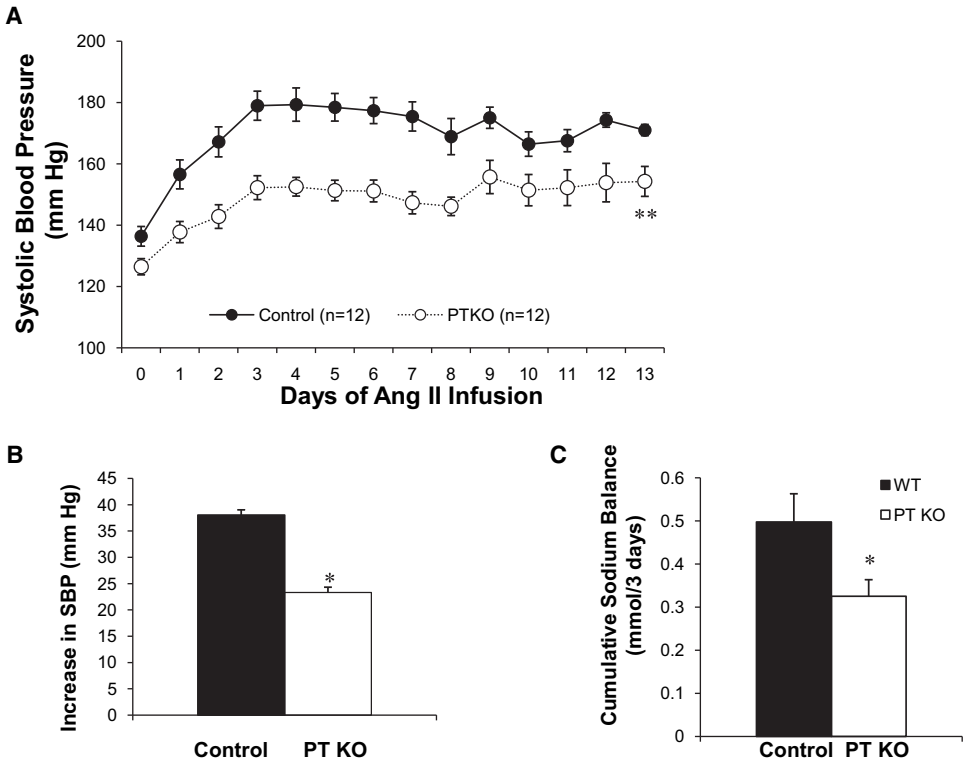

Figure 2. AT_{1A} Receptors in the Proximal Tubule Promote Hypertension

(A) With infusion of angiotensin II (Ang II, 1000 ng/kg/min), BPs increased significantly in both control and PTKO mice but the hypertensive response to angiotensin II was significantly attenuated in the PTKOs (**p < 0.001).

(B) The mean increase in BP during the angiotensin II infusion was significantly less in the PTKOs (23 ± 3 mm Hg) compared to controls (black bars; 38 ± 5 mm Hg, *p = 0.0005).

(C) Cumulative sodium balance was significantly lower in the PTKOs (n = 8) than controls (n = 7, *p = 0.046) during the first 3 days of Ang II infusion. Error bars represent SEM.

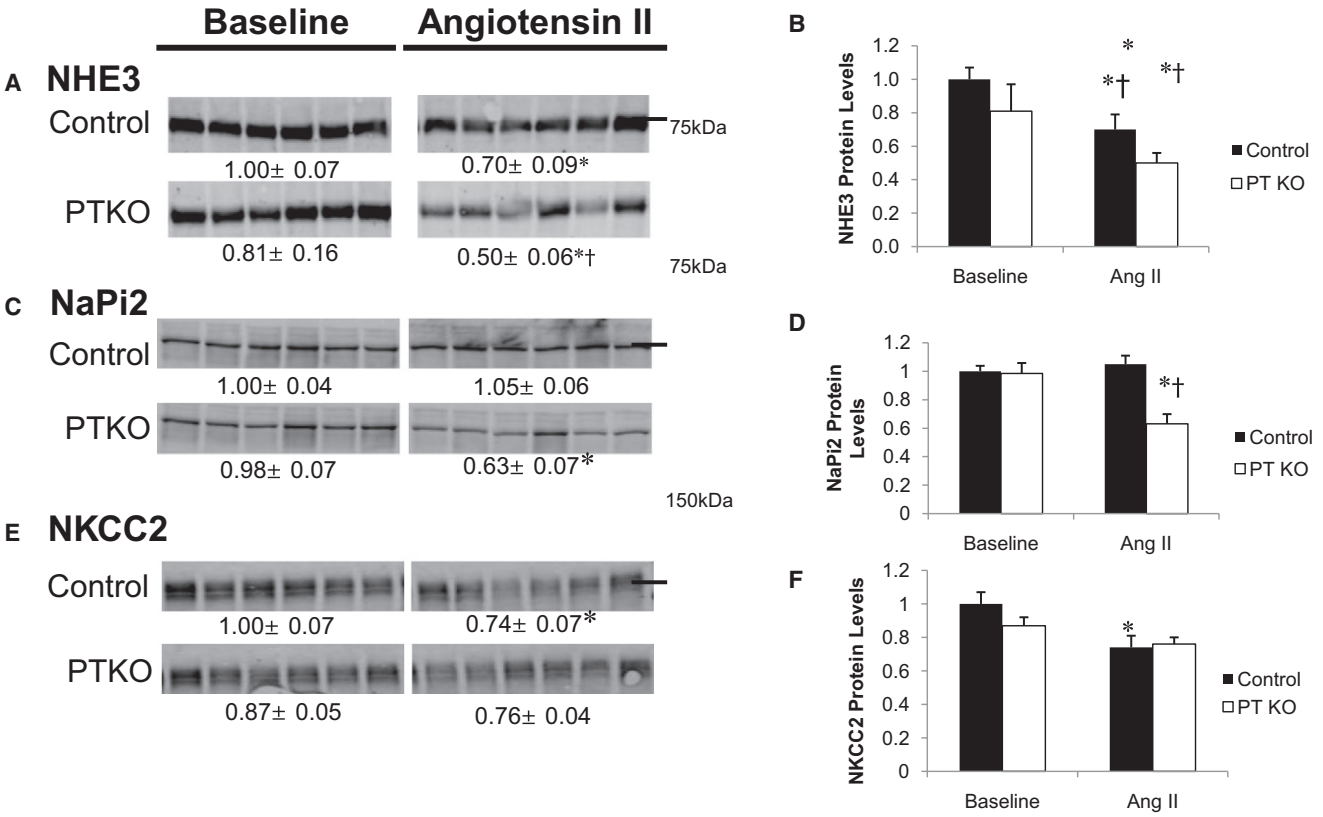

Figure 3. Abundance of Sodium Transporters in Control and PTKO Mice

Protein levels of key transporters in kidneys of PTKO and control mice including NHE3 (A and B), NaPi2 (C and D), and NKCC2 (E and F) were measured by western blot. Numbers below the blots indicate the mean ± SEM of the sample density normalized to the control baseline set (arbitrarily defined as 1.0). The densitometry data are in bar graphs (B, D, and F), where * indicates $p < 0.05$.

Abundances of the sodium-potassium-two-chloride cotransporter (NKCC2), a major sodium transporter downstream in the thick ascending limb of the loop of Henle, were equivalent at baseline in the two experimental groups (Figure 3) and fell to a similar extent during angiotensin II infusion (Figures 3E and 3F). Similarly, total protein abundance of the β subunit epithelial sodium channel (ENaCβ) expressed from late distal nephron through collecting ducts and the α1 subunit of the sodium-potassium ATPase (Na,K-ATPase) expressed all along the nephron were unaffected in PTKO mice (Figures S3A and S3B).

DISCUSSION

The importance of AT_1 angiotensin receptors in clinical medicine is highlighted by the impressive cardiovascular benefits of angiotensin receptor blockers (ARBs). As antihypertensive agents, these drugs are effective and well tolerated (Matchar et al., 2008), ameliorating morbidity and mortality associated with cardiovascular diseases (Brenner et al., 2001; Lewis et al., 2001; Pfeffer et al., 2003; Yusuf et al., 2008). By inference, these clinical studies indicate powerful contributions of AT_1 receptors to the pathogenesis of cardiovascular disease, hypertension, and kidney damage. However, the specific cellular targets responsible for their pathological actions cannot be identified from studies by using pharmacological inhibitors that block AT_1 receptors in all tissues.

The kidney has been suggested to play a predominant role in BP control. Guyton hypothesized that the substantial capacity of the kidney to excrete sodium provides a compensatory system of virtually infinite gain to oppose processes, including increases in peripheral vascular resistance, which would tend to increase BP (Guyton, 1991). This hypothesis is supported by the genetic studies of Lifton and associates linking Mendelian disorders impacting BP homeostasis to genetic variants affecting salt and water handling by the kidney (Lifton et al., 2001). On the other hand, a series of recent studies have suggested that vascular dysfunction alone may be sufficient to cause hypertension (Guilluy et al., 2010; Heximer et al., 2003; Michael et al., 2008; Wirth et al., 2008). For example, studies by Guilluy and associates indicate that elimination of *Arhgef1*, a Rho exchange factor linked to AT_1 receptor signaling, from smooth muscle apparently results in a complete abrogation of hypertension (Guilluy et al., 2010).

In previous studies, by using renal cross-transplantation we found distinct and significant roles for AT_{1A} receptor actions in both the kidney and extrarenal tissues in BP homeostasis (Crowley et al., 2005). In hypertension, however, receptors inside the kidney played the dominant role, driving elevations in BP as well as the development of cardiac hypertrophy (Crowley

et al., 2006). This was due to direct actions of AT_1 receptors in the renal parenchyma, independent of aldosterone. Within the kidney, AT_1 receptors are widely expressed in vasculature, in glomeruli, and by populations of epithelial cells across the nephron (Bouby et al., 1997). Activation of AT_1 receptors at any or all of these sites could potentially impact BP regulation.

Actions of angiotensin II to influence solute transport along the nephron have been well documented (Barreto-Chaves and Mello-Aires, 1996; Cogan, 1990; Geibel et al., 1990; Levine et al., 1996; Peti-Peterdi et al., 2002; Quan and Baum, 1998; Schuster et al., 1984; Wang et al., 1996). We considered that the population of AT_1 receptors in the proximal tubule may directly impact BP regulation because the major portion of the glomerular filtrate is reabsorbed here (Weinstein, 2008) and sodium transport by the proximal tubule may be a major determinant of the pressure-natriuresis response (McDonough et al., 2003). Furthermore, studies by Navar and Kobori have suggested that the proximal tubule is a key site of an independently regulated intrarenal RAS serving as a source of angiotensinogen and angiotensin II, which may influence nephron function (Kobori et al., 2007; Navar et al., 2002). The capacity for angiotensinogen generated in the proximal tubule to affect blood pressure was shown by Sigmund and associates in studies by using transgenic mice expressing human angiotensinogen and renin specifically in the proximal tubule (Lavoie et al., 2004). On the other hand, it has been suggested that the final adjustments of urinary sodium excretion in the distal nephron are also important for body fluid volume homeostasis (Meneton et al., 2004). In this regard, most of the human mutations with effects upon BP affect fluid reabsorption in the distal portion of the nephron (Lifton et al., 2001).

We generated mice with specific deletion of AT_{1A} receptors from epithelial cells in the proximal tubule by using the well-characterized PEPCK-*Cre* mouse line, which induces excision of floxed alleles from epithelial cells in the proximal but not distal tubule (Higgins et al., 2007; Rankin et al., 2006). We find that elimination of AT_{1A} receptors from the proximal tubule causes a significant reduction of baseline BP by $\approx$10 mm Hg. Notwithstanding recent reports of dominant actions of vascular signaling in BP control (Guilluy et al., 2010; Heximer et al., 2003; Michael et al., 2008; Wirth et al., 2008), the low BPs in the PTKOs occurred despite intact constrictor responses to angiotensin II in the peripheral vasculature. This nonredundant role for AT_1 receptors in the proximal tubule to determine BP level also suggests there is tonic stimulation of these receptors in the basal, euvolemic state consistent with previous studies showing that acute administration of specific AT_1 receptor blockers to rats inhibits net proximal reabsorption (Thomson et al., 2006; Xie et al., 1990). Similarly, we find that elimination of AT_{1A} receptors from the proximal tubule also reduces rates of fluid reabsorption.

Agtr1a$^{-/-}$ mice with global deletion of AT_{1A} receptors have exaggerated fluctuations in BP with extremes of dietary sodium intake (sodium sensitivity) (Oliverio et al., 2000). Enhanced sodium sensitivity is associated with absence of AT_{1A} receptors from the kidney, whereas elimination of receptors only from extrarenal tissues does not affect this response (Crowley et al., 2005). Despite their lower basal BPs, the magnitude of BP changes during high- and low-salt feeding was very similar in PTKOs and controls (Figure 1C). Thus, deletion of AT_{1A} receptors from the proximal tubule alone is not sufficient to generate a phenotype of enhanced sodium sensitivity, indicating that AT_1 receptor pools at other sites, perhaps in the distal nephron or renal vasculature, may control this response.

We find that elimination of AT_1 receptors from the proximal tubule provides significant protection against angiotensin II-dependent hypertension, identifying this epithelial compartment as a target of the RAS that is critical to the pathogenesis of hypertension. Protection from hypertension is associated with enhanced natriuresis and altered sodium balance, suggesting that modulation of sodium handling is critical for these actions. As discussed above, activation of AT_1 receptors stimulates fluid reabsorption in proximal tubules (Schuster et al., 1984; Cogan, 1990) by triggering coordinate stimulation of the luminal sodium-proton antiporter (NHE3) along with the basolateral Na,K-ATPase (Cogan, 1990; Geibel et al., 1990). Levels and localization of key sodium transporters in the proximal tubule are modified during ACE inhibition, suggesting control of their synthesis and cell trafficking by angiotensin II (Yang et al., 2007).

We examined the contributions of AT_1 receptors in the proximal tubule in isolation to regulate abundance of key sodium transporters in vivo in the intact animal. Under basal conditions, there were no statistically significant differences in major transporter abundance between the groups, although the levels of NHE3 tended to be lower in the PTKOs. In this setting, the PTKO animals are in balance and their urinary excretion of sodium reflects dietary intake. Therefore, it follows that their transporter profiles would not differ dramatically from controls. Further, because of their reduced BPs and GFRs, hemodynamic factors such as changes in peritubular capillary pressures may also influence sodium reabsorption in the PTKOs independent of absolute levels of epithelial transporters. By contrast, the regulation of luminal transporters by AT_{1A} receptors in the proximal tubule is clearly revealed when the steady state is abruptly modified during chronic infusion of angiotensin II. With angiotensin II infusion there was a significant reduction in NHE3 within the control group compared to baseline. Such attenuation of NHE3 expression in response to BP elevation has been described previously (McDonough et al., 2003) and may be one mechanism facilitating natriuresis as pressure increases. The further and significant reduction in NHE3 levels in the PTKOs compared to controls is consistent with a lower threshold for pressure natriuresis in these mice, supported by the differences we observed in net sodium balance (Figure 2C). We also found that NaPi2 expression during angiotensin II infusion is regulated by AT_{1A} receptors in the proximal tubule (Figure 3). Taken together, these findings provide clear evidence for actions of AT_{1A} receptors in the proximal tubule to oppose adaptive reductions in key sodium transporters in the proximal tubule during hypertension, with the net effect of impairing the pressure natriuresis response (Magyar and McDonough, 2000). When this pathway is eliminated, the hypertensive response is attenuated (Figures 2A and 2B), highlighting the power of mechanisms controlling renal solute reabsorption in the proximal tubule to determine BP levels.

In summary, our studies show that the epithelium of the proximal tubule is a critical location for integrating signals to set the level of intra-arterial pressure. This is accomplished though

modulation of tubular fluid reabsorption and regulated expression of sodium transporter proteins. The RAS provides tonic control of this pathway supporting BP independent of its vascular actions. Furthermore, it is likely that other neurohormonal systems exploit these proximal tubular mechanisms for physiological control (DiBona, 2005; Zeng et al., 2009; Zhang et al., 2009). Our data suggest that effective inhibition of sodium reabsorption in the renal proximal tubule could be a useful therapeutic strategy in hypertension, supplementing the range of diuretic agents currently used to target sodium reabsorption in distal nephron segments. Our studies further suggest that blockade of this pathway is probably a key component underlying the therapeutic efficacy of RAS antagonists in the treatment of hypertension.

EXPERIMENTAL PROCEDURES

Animals

Inbred 129/SvEv mice with a conditional *Agtr1a* allele were generated as described in Supplemental Experimental Procedures. The *Pepck-Cre* transgene was backcrossed more than 10 generations onto the 129/SvEv background. Mice were bred and maintained in the animal facility at the Durham VA Medical Center according to NIH guidelines.

BP Measurements in Conscious Mice

BPs were measured in conscious mice by using radiotelemetry as described previously (Crowley et al., 2005).

Sodium Balance Studies

Measurements of sodium balance were carried out as described previously (Crowley et al., 2006) with individual metabolic cages and a gel diet containing nutrients, water, and 0.1% w/w sodium (Nutra-Gel; Bio-Serv, Frenchtown, NJ).

Micropuncture Studies

Mice were anesthetized and prepared for micropuncture studies as described previously (Hashimoto et al., 2005). To determine nephron filtration and absorption rates, we infused ^{125}I iothalamate (Glofil, Questcor Pharmaceuticals, Hayward, CA) at ~40 μCi/hr. A blood sample was collected after 20–25 min of equilibration and free-flow micropuncture was performed.

Immunoblot Analysis

Levels of renal transporter proteins were determined as described previously (Yang et al., 2007). Kidneys were decapsulated, individually homogenized, and centrifuged and the supernatant homogenate was retained. A constant amount of homogenate protein from each animal was denatured, resolved on 7.5% SDS-polyacrylamide gels, and transferred to a single polyvinylidene difluoride membrane (Millipore Immobilon-P). After blocking, blots were incubated with specific antibodies (listed in Supplemental Experimental Procedures). Sample amounts were determined to be in the linear range of detection and signals were quantitated with the Odyssey Infrared Imaging System (LI-COR, Lincoln, NE). Data for each protein were normalized to the mean of expression in the control noninfused group defined as 1.0.

Statistical Analysis

The values for each parameter within a group are expressed as the mean ± the standard error of the mean (SEM). For comparisons between groups, statistical significance was assessed by using ANOVA followed by unpaired t test adjusted for multiple comparisons (normally distributed data) and the Mann-Whitney U test was employed for nonparametric data. For comparisons within groups, normally distributed variables were analyzed by a paired t test, whereas nonparametric analysis was with the Wilcoxon signed rank test. Normality was determined by using the Shapiro-Wilk W test.

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures, one table, and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.cmet.2011.03.001.

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