Cell Metabolism
Short Article

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

Susan B. Gurley,[1] Anne D.M. Riquier-Brison,[2] Jurgen Schnermann,[3] Matthew A. Sparks,[1] Andrew M. Allen,[4] Volker H. Haase,[5] John N. Snouwaert,[6] Thu H. Le,[7] Alicia A. McDonough,[2] Beverley H. Koller,[6] and Thomas M. Coffman[1,8,*]
[1]Division of Nephrology, Department of Medicine, Duke University and Durham, VA Medical Centers, Durham, NC 27710, USA
[2]Department of Cell and Neurobiology, University of Southern California, Los Angeles, CA 90089, USA
[3]National Institute of Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA
[4]Department of Physiology, University of Melbourne, Melbourne, Victoria 3010, Australia
[5]Division of Nephrology and Hypertension, Department of Medicine, Vanderbilt University, Nashville, TN 37232, USA
[6]Department of Genetics, University of North Carolina, Chapel Hill, NC 27599, USA
[7]Division of Nephrology, Department of Medicine, University of Virginia, Charlottesville, VA 22908, USA
[8]Cardiovascular and Metabolic Disorders Research Program, Duke-NUS Graduate Medical School, Singapore 169857
*Correspondence: tcoffman@duke.edu
DOI 10.1016/j.cmet.2011.03.001

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

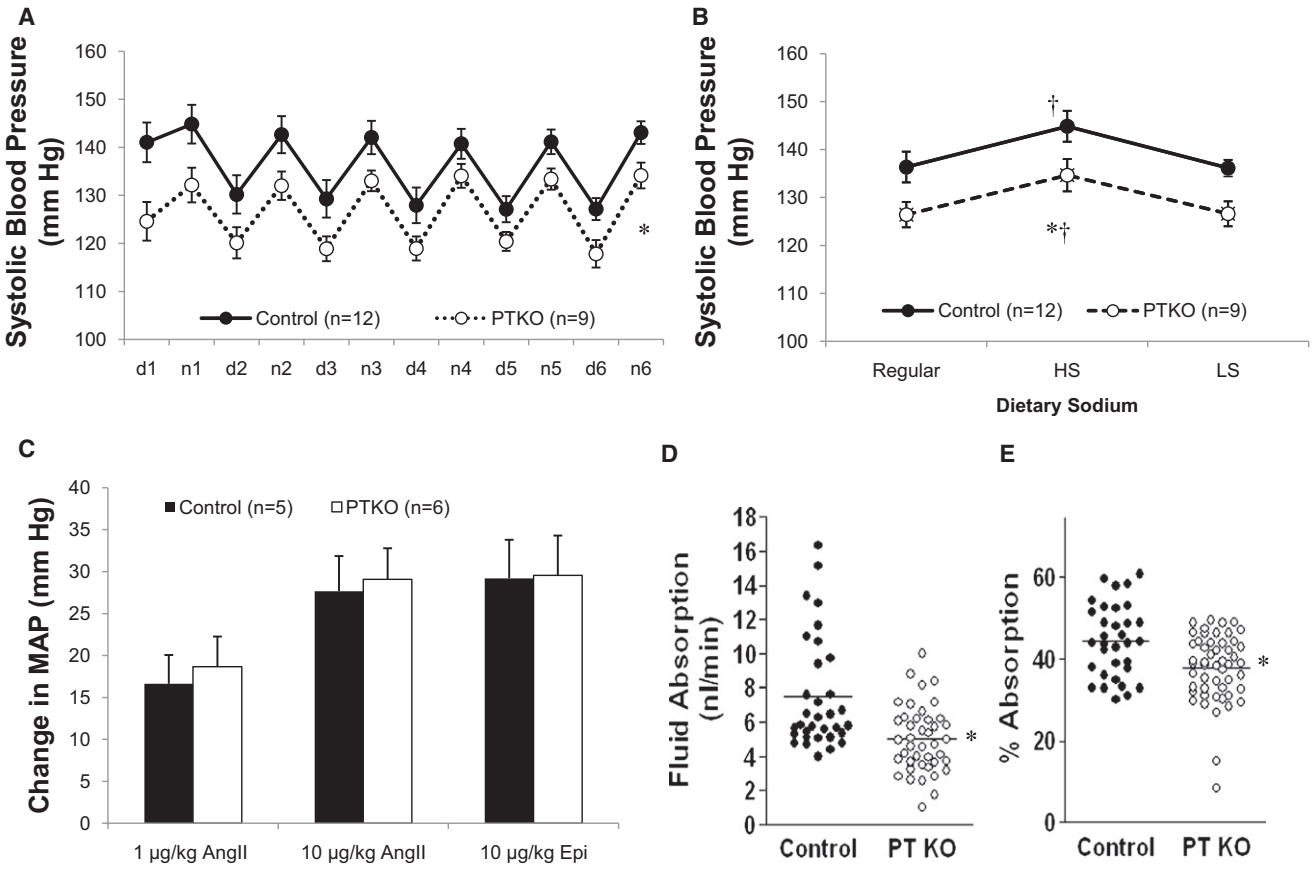

Figure 1. Baseline Studies in PTKO Mice

(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