Elastin insufficiency causes hypertension, structural defects and abnormal remodeling of renal vascular signaling

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Elastin deficiency causes vascular stiffening, a leading risk for hypertension and chronic kidney disease (CKD). The mechanisms mediating hypertension and/or CKD pathogenesis due to elastin deficiency are poorly understood. Using the elastin heterozygous (*Eln*+/−) mouse model, we tested whether renal dysfunction due to elastin deficiency occurs independently of and precedes the development of hypertension. We assessed blood pressure and renal hemodynamics in 30-day and 12-week-old male and female mice. At P30, blood pressure of *Eln*+/− mice was similar to wild-type controls; however, renal blood flow was lower, whereas renal vascular resistance was augmented at baseline in *Eln*+/− mice. At 12 weeks, renal vascular resistance remained elevated while filtration fraction was higher in male *Eln*+/− relative to wild-type mice. Heterozygous mice showed isolated systolic hypertension that was evident only at nighttime. Acute salt loading with 6% dietary sodium increased daytime systolic blood pressure only in male *Eln*+/− mice, causing a rightward shift and blunted slope of the pressure-natriuresis curve. Renal interlobar artery basal tone and myogenic response to increasing intraluminal pressure at day 10 were similar, whereas they were augmented at day 30 and at 12 weeks old in *Eln*+/− mice, and normalized by the AT1R blocker, candesartan. Heterozygous mice also exhibited podocyte foot process damage that persisted even when blood pressure was normalized to wild-type levels with hydralazine. Thus, elastin insufficiency triggers structural defects and abnormal remodeling of renal vascular signaling involving AT1R-mediated vascular mechanotransduction and renal hyperfiltration with increased blood pressure sensitivity to dietary sodium contributing to systolic hypertension.

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The key regulatory mechanisms that maintain long-term blood pressure homeostasis depend on the control of tone in the resistance vasculature and the kidney's ability to regulate cardiac output by maintaining extracellular fluid volume and electrolyte balance. The kidney's ability to reabsorb or excrete excess water and sodium is the principal mechanism that integrates the regulation of extracellular fluid volume, plasma osmolality, cardiac output, and systemic blood pressure.[1] Accordingly, changes in the circulating levels of hormones such as angiotensin II from the renin-angiotensin system, or the expression or activity or both of their target receptors that stimulate thirst or promote sodium reabsorption or both can alter homeostatic blood pressure.[1,2] Furthermore, structural damage to the nephron, as occurs in barotraumatic insult to the glomerular filtration barrier, can trigger compensatory yet maladaptive increases in renal tubular reabsorption of water and sodium that further exacerbate the preceding blood pressure abnormality.[3–5]

Under normal physiology, the extrinsic and intrinsic factors that moderate renal vascular resistance (RVR) maintain constant renal blood flow (RBF) and glomerular filtration rate (GFR) within a certain blood pressure range by preventing excessive vasoconstriction that could potentially cause renal ischemia or the transmission of high systemic blood pressure causing barotraumatic insult to the glomerular filtration barrier.[6–8] This moderating phenomenon, termed renal autoregulation, involves mechanisms that cause vasoconstriction or dilatation of renal preglomerular arteries in response to fluctuations in systemic blood pressure or sodium sensing by the macula densa.[8,9] Sustained elevation of systemic blood pressure, however, evokes a state of chronically elevated RVR that can cause GFR reduction due to renal hypoperfusion.[10] Conversely, defective renal autoregulation and low RVR permits the transmission of systemic blood pressure to the kidney, where it can cause glomerular barotrauma and damage to the glomerular filtration barrier, resulting in hyperfiltration, proteinuria, and altered sodium excretion and reabsorption by the renal tubular system, all of which are hallmarks of chronic kidney disease (CKD).[11,12] Therefore, physiological mechanisms involved in the

regulation of vascular function and thus renal vascular tone are promising targets to control high blood pressure, which is a leading cause of CKD.[13,14]

Mice deficient in elastin, an extracellular matrix (ECM) protein that confers compliance to conduit vessels, develop hypertension that has been attributed mostly to structural abnormalities in the aorta and other conduit vessels and hyperactivity of the renin-angiotensin system.[15,16] An intriguing observation made in the early assessment of the cardiovascular phenotypes of elastin heterozygous ($Eln^{+/-}$) mice was that abnormal changes in the mechanical properties of conduit vessels, specifically the aorta, occur within a few days after birth and precede the detection of any measureable change in systemic blood pressure.[17] This observation led us to hypothesize that hypertension in $Eln^{+/-}$ mice is a consequence of maladaptive remodeling of organ systems that regulate blood pressure determinants. Recently, we reported that structural and functional impairment in resistance arteries of the splanchnic vascular bed contributes to elevated blood pressure in $Eln^{+/-}$ mice and involves hyperactivity of the angiotensin type 1 receptor (AT1R).[16] However, what remains unresolved are the effects of elastin insufficiency on renal structure and function and whether those effects play a role in the development of hypertension.

To address these questions, we examined the mechanical and functional properties of small interlobar arteries of the kidney in wild-type (WT) and $Eln^{+/-}$ mice at postnatal days (PDs) 10 and 30 when blood pressure is normal[17] and at 12 weeks of age when hypertension due to *Eln* insufficiency is fully established.[15] Our study shows that elastin deficiency age-dependently alters renal hemodynamics and blood pressure sensitivity to high sodium intake and that these changes are exacerbated by the ensuing hypertension. The findings are highly relevant to understanding the role of elastin deficiency in the development of hypertension and CKD.

RESULTS

Increased renal vascular tone precedes hypertension development in $Eln^{+/-}$ mice

Renal dysfunction is a hallmark of hypertension, which often involves aberrant activity of the renin-angiotensin system (RAS), including abnormal levels of angiotensin II and increased activity of contractile angiotensin receptors.[18–20] In $Eln^{+/-}$ mice, the development of hypertension has been attributed partly to increased RAS activity.[15] Accordingly, pharmacological blockade of the RAS at the receptor level, or several steps downstream of the AT1R signaling pathway, normalizes blood pressure and decreases vascular reactivity to G protein–coupled receptor (GPCR) activation in $Eln^{+/-}$ mice.[15,16,21] Whether renal dysfunction plays a causal role or, is a consequence of sustained hypertension in elastin-insufficient mice is not known. To address this question, we determined whether renal dysfunction precedes the onset of hypertension in $Eln^{+/-}$ mice. First, we determined whether arteriopathy arising from elastin insufficiency extended into the renal vascular bed. We found that the abnormal vascular morphologies, including elongated and tortuous appearance noted in conduit vessels of $Eln^{+/-}$ mice,[17,22] were evident in the renal vasculature and are visible as early as PD10, becoming more prominent with age (Figure 1). We also observed that, in contrast to the splanchnic vascular bed, where elastin insufficiency leads to the appearance of extra-medial elastic lamina,[16] in the kidney of elastin-deficient mice, both the internal and external elastic laminae are thinner and less defined or visible compared with WT arteries (Figure 1b). Next, we assessed RBF, RVR, and GFR in mice shortly after weaning, that is, at PD30. At this age, we found that baseline blood pressure levels and GFR were similar between WT and $Eln^{+/-}$ mice (Figure 2a), whereas RBF was decreased while RVR was elevated in $Eln^{+/-}$ mice relative to WT control mice (Figure 2b–d). We then assessed preglomerular vascular tone by measuring myogenic response of renal interlobar arteries at PD10 and PD30 using *ex vivo* vessel preparation approach. At PD30, both basal tone and myogenic response to increasing intraluminal pressure, calculated using vessel diameter in the presence and absence of calcium (Figure 3a and b), were markedly augmented in arteries from $Eln^{+/-}$ mice relative to WT arteries (Figure 3c). At intraluminal pressures of 120 and 140 mm Hg, percentage of myogenic tone began to fall as the vessels force-dilated, suggesting that, at this postnatal age, these are likely the upper limits of pressure range within which myogenic constriction operates. Despite increased myogenic constriction in $Eln^{+/-}$ mice, wall tension in the presence or absence of calcium was similar at all pressures in both genotypes (Figure 3e). However, medial thickness-to-lumen diameter ratio was higher in interlobar arteries from $Eln^{+/-}$ relative to WT mice (Figure 3f), while medial cross-sectional area remained unchanged (data not shown). In contrast, basal tone and myogenic response at PD10 were similar between WT and $Eln^{+/-}$ mice (Figure 3d). Together, these results indicated that the onset of structural and functional abnormalities of the renal vasculature precede hypertension development in $Eln^{+/-}$ mice.

Augmented vascular tone in renal interlobar arteries persists in adult $Eln^{+/-}$ mice and is mediated by agonist-independent activity of AT1Rs

Several studies utilizing blood vessels of different diameters have established that arteriopathy, due to both structural and functional remodeling, contributes to hypertension development as a result of elastin insufficiency.[15–17,23,24] However, the mechanism by which elastin deficiency translates to functional stiffening of resistance arteries has not been defined. To fill this gap in knowledge, we took advantage of current hypothesis in the field that certain putative receptors and membrane proteins such as AT1R, vascular epithelial sodium channel, acid-sensing ion channel, and integrins are capable of detecting changes in ECM stiffness and transduce such stimuli via mechanotransduction.[25–28] We focused on