ORIGINAL ARTICLE

Cytosolic Phospholipase A$_2$α Is Essential for Renal Dysfunction and End-Organ Damage Associated With Angiotensin II-Induced Hypertension

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BACKGROUND
The kidney plays an important role in regulating blood pressure (BP). cPLA2α in the kidney is activated by various agents including angiotensin II (Ang II) and selectively releases arachidonic acid (AA) from tissue lipids, generating pro- and antihypertensive eicosanoids. Since activation of cPLA2α is the rate-limiting step in AA release, this study was conducted to determine its contribution to renal dysfunction and end-organ damage associated with Ang II-induced hypertension.

METHODS
cPLA$_2$α$^{+/+}$ and cPLA$_2$α$^{-/-}$ mice were infused with Ang II (700 ng/kg/min) or its vehicle for 13 days. Mice were placed in metabolic cages to monitor their food and water intake, and urine was collected and its volume was measured. Doppler imaging was performed to assess renal hemodynamics. On the 13th day of Ang II infusion, mice were sacrificed and their tissues and blood collected for further analysis.

RESULTS
Ang II increased renal vascular resistance, water intake, and urine output and Na$^+$ excretion, decreased urine osmolality, and produced proteinuria in cPLA$_2$α$^{+/+}$ mice. Ang II also caused accumulation of F4/80$^+$ macrophages and CD3$^+$ T cells and renal fibrosis, and increased oxidative stress in the kidneys of cPLA$_2$α$^{+/+}$ mice. All these effects of Ang II were minimized in cPLA$_2$α$^{-/-}$ mice.

CONCLUSION
cPLA$_2$α contributes to renal dysfunction, inflammation, and end-organ damage, most likely via the action of pro-hypertensive eicosanoids and increased oxidative stress associated with Ang II-induced hypertension. Thus, cPLA$_2$α could serve as a potential therapeutic target for treating renal dysfunction and end-organ damage in hypertension.

Keywords: angiotensin II; blood pressure; cPLA2α$^{-/-}$ mice; fibrosis; hypertension; inflammation; oxidative stress; proteinuria; renal hemodynamics.

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Kidney plays an important role in regulating blood pressure (BP) by maintaining Na$^+$ and extracellular fluid homeostasis. Alteration in kidney function promotes the development of hypertension and end-stage organ damage.[1] The renin-angiotensin system contributes to the regulation of Na$^+$ and extracellular fluid homeostasis and long-term maintenance of arterial BP via generation of angiotensin II (Ang II).[1,2] Kidney also contains cPLA$_2$[3] that releases arachidonic acid (AA) from tissue lipids[4] in response to various agents including Ang II.[5] AA is metabolized via cyclooxygenases (COXs) into prostaglandins (PG) E2, F2$_α$, D2, I2, and thromboxane (Tx) A2[6], by lipoxygenases into leukotrienes and 5-, 12-, and 15-hydroxyeicosatrienoic acids (HETE),[7] by cytochrome P450 4A through ω-hydroxylase into 20-HETE, and by epoxygenase into epoxyeicosatrienoic acids.[8–10] These eicosanoids produce diverse actions and contribute to renal function as well as to the pathogenesis of renal dysfunction in various kidney diseases.[11]

Many of the eicosanoids generated from AA modulate and/or mediate one or more biological actions of Ang II.[12] PGE2, PGI2, and epoxyeicosatrienoic acids decrease vascular tone and/or promote salt/water excretion and minimize vascular actions of Ang II,[6,8–13] thus contributing to an antihypertensive mechanism. The vasodepressor effects of PGE2 are mediated via prostaglandin E (EP) 2 and EP4 receptors.[14] However, PGE2 via EP1 and EP3 receptors and TxA2 via Thromboxane (TP) receptor produce vasopressor effects and contribute to Ang II-induced hypertension.[14–16] 12- and 20-HETE also mediate vascular effects of Ang II and contribute to its pro-hypertensive mechanism,[17,18] and by increasing salt and water excretion via their tubular effects contributes to antihypertensive mechanisms.[10] Cytochrome P450 1B1, expressed in cardiovascular and renal tissues, can metabolize various substrates including AA and contributes to renal dysfunction associated with Ang II-induced hypertension by generating reactive

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oxygen species (ROS) in male mice.[19] Therefore, the release of AA by cPLA$_2$ from tissue lipids, the rate-limiting step in the biosynthesis of eicosanoids, could be a major determinant of the cardiovascular and renal actions of Ang II. Among six cPLA$_2$ isoforms, cPLA$_2$α has been well studied and plays a major role in AA release. Recently, we showed that cPLA$_2$α is critical for the development of Ang II-induced hypertension and associated cardiovascular pathogenesis.[20] The present study was conducted to test the hypothesis that cPLA$_2$α is also crucial for renal dysfunction, end-organ damage, and inflammation. The results of our study show that cPLA$_2$α gene disruption in male mice ameliorates renal dysfunction, end-organ damage, and inflammation associated with Ang II-induced hypertension most likely due to diminished production of pro-hypertensive eicosanoids and lipid peroxides.

METHODS

The detailed experimental methods are described in the Supplementary Data.

RESULTS

cPLA$_2$α gene disruption prevents Ang II-induced increase in cPLA$_2$ activity and renal vascular resistance

cPLA$_2$α gene disruption selectively prevents its expression without altering the expression of other related lipases.[20]

Ang II infusion increased renal cPLA$_2$ activity as measured by its phosphorylation without altering its expression in cPLA$_2$α$^{+/+}$ mice. As expected, protein expression of cPLA$_2$ was not observed in the cPLA$_2$α$^{-/-}$ mice (Supplementary Figure S1). Recently we reported that Ang II increases BP and associated cardiac dysfunction in cPLA$_2$α $^{+/+}$ but not cPLA$_2$α$^{-/-}$ mice.[20] In these mice the effect of Ang II on renal hemodynamics was determined by pulse-wave Doppler method. A representative ultrasound B mode image of the kidney in transverse view is shown in Figure 1A, color Doppler to visualize blood flow in Figure 1B, and pulse-wave Doppler mode in Figure 1C. Renal artery resistive and pulsatility index were calculated as a measure of resistance and variability of blood velocity in the renal artery. Ang II infusion for 13 days increased renal vascular resistance (Figure 1D) and pulsatility index (Figure 1E) in cPLA$_2$α$^{+/+}$ but not in cPLA$_2$α$^{-/-}$ mice.

cPLA$_2$α gene disruption minimizes Ang II-induced renal dysfunction

Water intake and urine output were not different between cPLA$_2$α$^{+/+}$ and cPLA$_2$α$^{-/-}$ mice. Infusion of Ang II for 13 days increased water intake (Figure 2A) and urine output (Figure 2B), decreased urine osmolality (Figure 2C), did not alter plasma creatinine levels, an index of glomerular filtration rate (Figure 2D), and increased urinary Na$^+$ excretion (Figure 2E) and caused proteinuria (Figure 2F) in
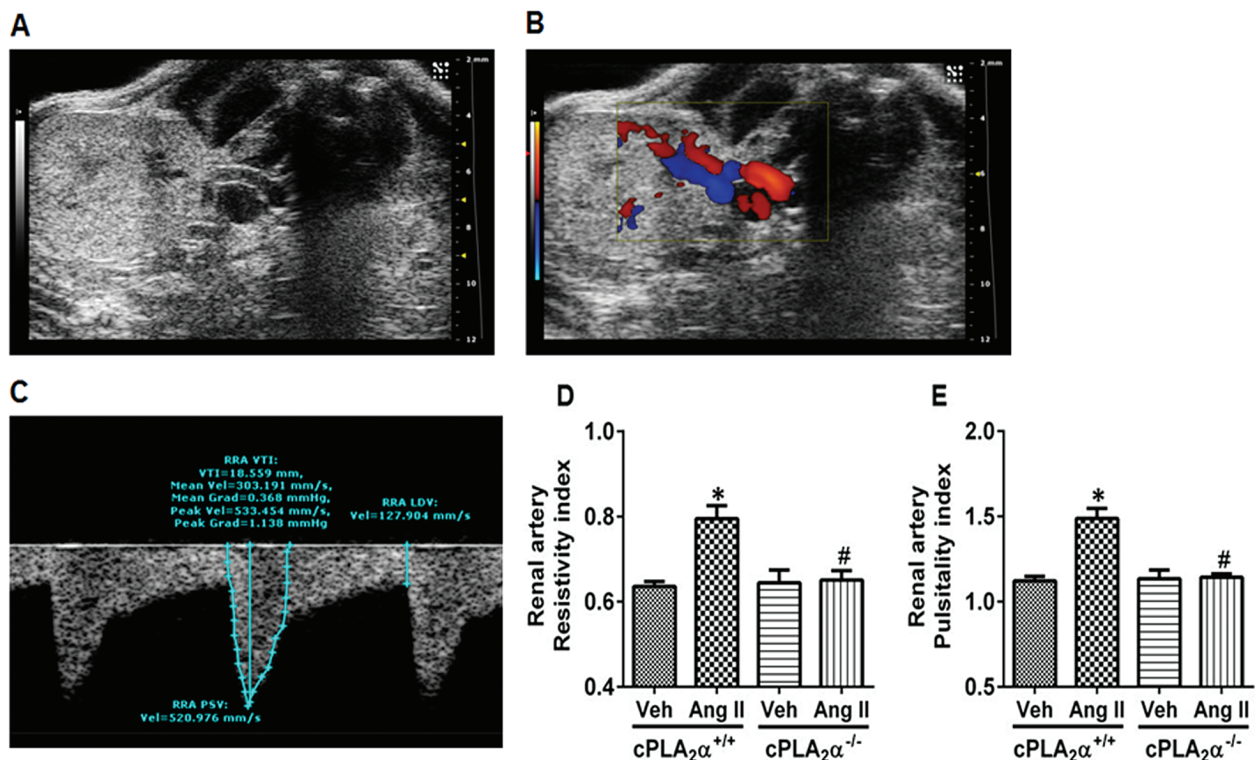

Figure 1. Angiotensin II (Ang II) infusion alters renal hemodynamics in cPLA$_2$α$^{+/+}$, But Not cPLA$_2$α$^{-/-}$, Mice. Representative ultrasound B mode image of the kidney in transverse view (**A**), color Doppler to visualize blood flow (**B**), pulse-wave Doppler mode (**C**), renal artery resistive index (**D**), and renal artery pulsatility index (**E**).*$P < 0.05$ cPLA$_2$α$^{+/+}$ vehicle vs. cPLA$_2$α$^{+/+}$ Ang II, #$P < 0.05$ cPLA$_2$α$^{+/+}$ Ang II vs. cPLA$_2$α$^{-/-}$ Ang II ($n = 3$–6 for each group, data are expressed as mean ± SEM).
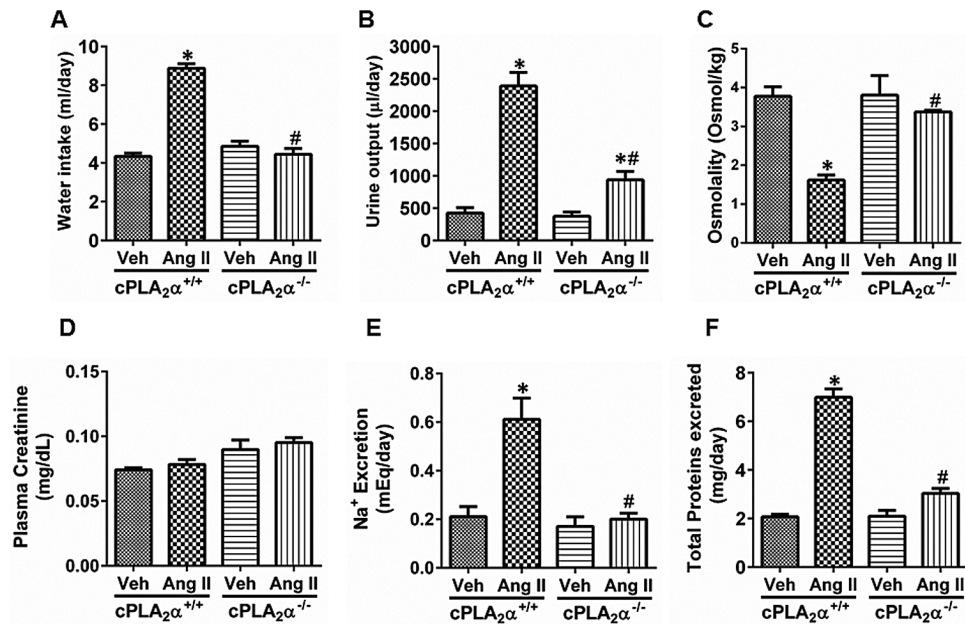
Figure 2. cPLA$_2$α gene disruption minimizes angiotensin II (Ang II)-induced renal dysfunction. Water intake (**A**), urine output (**B**), urine osmolality (**C**), plasma creatinine (**D**), Na$^+$ excretion (**E**), and protein excretion in the urine (**F**) were measured on day 13 of Ang II infusion, *$P < 0.05$ cPLA$_2$α$^{+/+}$ vehicle vs. cPLA$_2$α$^{+/+}$ Ang II, #$P < 0.05$ cPLA$_2$α$^{+/+}$ Ang II vs. cPLA$_2$α$^{-/-}$ Ang II ($n = 6$–8 for each group, data are expressed as mean ± SEM).

cPLA$_2$α$^{+/+}$ mice; these changes were minimized in cPLA$_2$α$^{-/-}$ mice except that the plasma creatinine level was not different from that observed in cPLA$_2$α$^{+/+}$ mice (Figure 2E).

cPLA$_2$α gene disruption prevents renal inflammation

To determine the contribution of cPLA$_2$α to inflammation associated with Ang II-induced end-organ damage, we examined the localization of CD3$^+$ T lymphocytes and F4/80$^+$ macrophages in the renal tissue of cPLA$_2$α$^{+/+}$ and cPLA$_2$α$^{-/-}$ mice. Ang II caused accumulation of CD3$^+$ T cells (Figure 3A) and infiltration of F4/80$^+$ macrophages (Figure 3B) in the glomerulus of cPLA$_2$α$^{+/+}$ but not cPLA$_2$α$^{-/-}$ mice.

cPLA$_2$α gene disruption prevents renal fibrosis

Increased interstitial staining of α-smooth muscle actin (Figure 4A) and transforming growth factor-β (Figure 4B), contributors of fibrosis, were observed in renal sections from Ang II-infused cPLA$_2$α$^{+/+}$, but not in cPLA$_2$α$^{-/-}$ mice. Increased collagen accumulation was observed in the interstitial space in the kidney of Ang II-infused cPLA$_2$α$^{+/+}$ but not cPLA$_2$α$^{-/-}$ mice (Figure 4C).

cPLA$_2$α gene disruption protects against Ang II-induced increase in renal oxidative stress

Ang II infusion for 13 days increased NADPH oxidase activity (Figure 5A) and thiobarbituric acid reactive substances (Figure 5B), a by-product of lipid peroxidation in cPLA$_2$α$^{+/+}$ but not in cPLA$_2$α$^{-/-}$ mice. These data correlated with increased superoxide production in renal sections of

cPLA$_2$α$^{+/+}$ mice, as indicated by increase in 2-hydroxyethidium fluorescence intensity (Figure 5C, D). This increase was not observed in cPLA$_2$α$^{-/-}$ mice infused with Ang II.

cPLA$_2$α gene disruption does not alter plasma levels of endothelin

Since endothelin has been implicated in Ang II-induced hypertension and some of its renal actions,[21,22] we measured the plasma levels of endothelin. Ang II infusion increased plasma levels of endothelin equally in both cPLA$_2$α$^{+/+}$ and cPLA$_2$α$^{-/-}$ mice, but the increase was not statistically significant (Supplementary Figure S2).

cPLA$_2$α gene disruption reduces mRNA expression of AT1$_A$, AT2, and Mas (Mas 1 protooncogene, G-protein-coupled) receptors, angiotensin-converting enzyme (ACE) and ACE2 in the kidney

To determine if Ang II-induced renal dysfunction, damage, and inflammation in cPLA$_2$α$^{+/+}$ but not in cPLA$_2$α$^{-/-}$ mice are due to alterations in one or more components of the renin-angiotensin system, we examined renal expression of AT1$_A$, AT2, Mas receptors, ACE, and ACE2. In each case the basal mRNA expression was not different in the kidneys of cPLA$_2$α$^{+/+}$ and cPLA$_2$α$^{-/-}$ mice. Ang II infusion increased mRNA expression of AT1$_A$R (Supplementary Figure S3A) and AT2R (Supplementary Figure S3B) receptors in cPLA$_2$α$^{+/+}$ but not cPLA$_2$α$^{-/-}$ mice. Ang II also increased Mas receptor mRNA expression, although it did not reach statistical significance in cPLA$_2$α$^{+/+}$ mice. Mas receptor mRNA was diminished in cPLA$_2$α$^{-/-}$ compared to cPLA$_2$α$^{+/+}$ mice (Supplementary Figure S3C). Expression
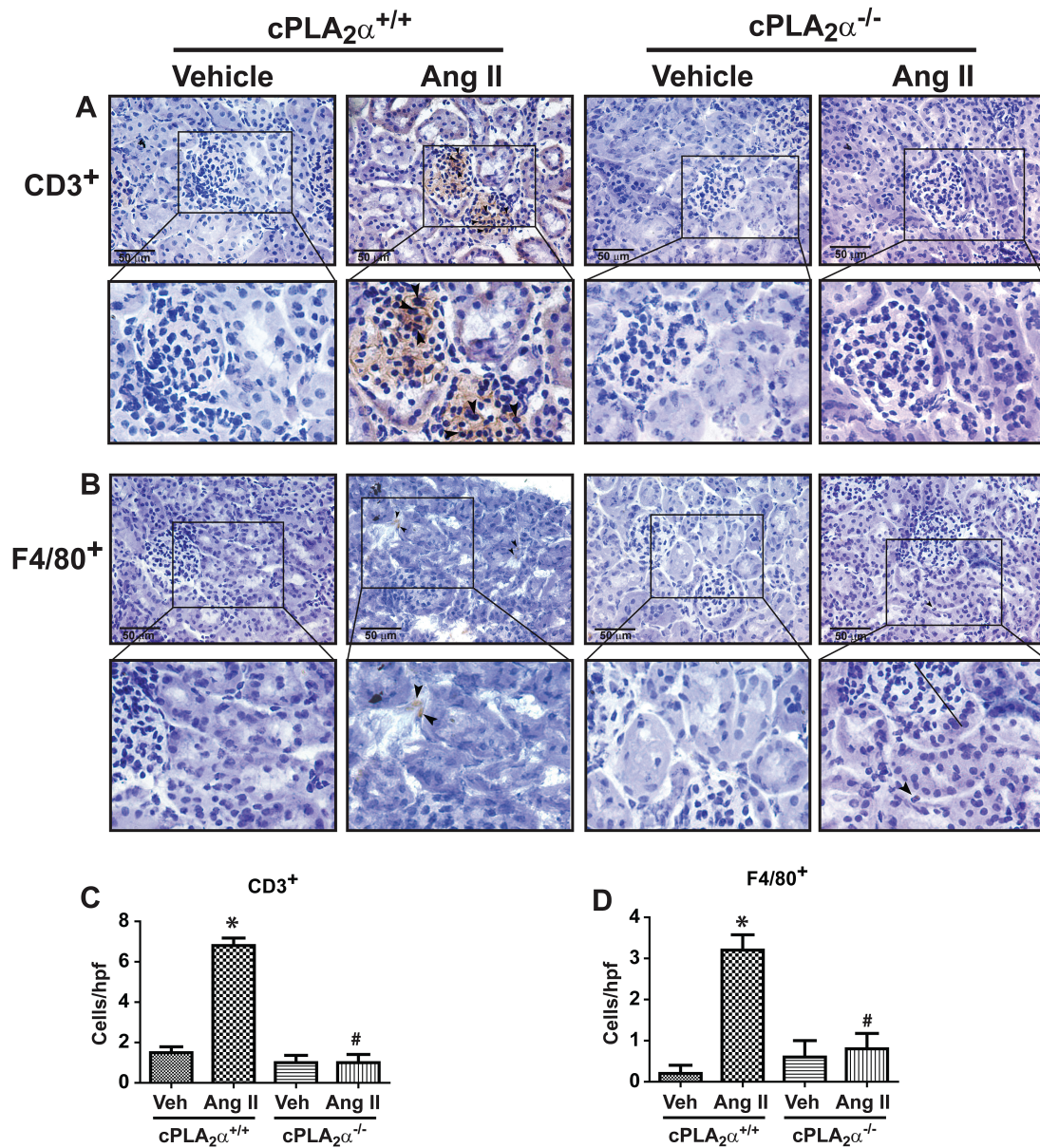
Figure 3. cPLA$_2$α gene disruption prevents renal inflammation. CD3$^+$ T cell (**A**) and F4/80$^+$ macrophages (**B**) in glomerulus of the kidney, as determined by immunohistochemical analysis. Panels (**C**) and (**D**) represent quantified data as cells/high power field (hpf). *$P < 0.05$ cPLA$_2$α$^{+/+}$ vehicle vs. cPLA$_2$α$^{+/+}$ Ang II, #$P < 0.05$ cPLA$_2$α$^{+/+}$ Ang II vs. cPLA$_2$α$^{-/-}$ Ang II ($n = 4$ for each group, data are expressed as mean ± SEM).

of ACE mRNA was decreased in cPLA$_2$α$^{+/+}$ mice and was further reduced in cPLA$_2$α$^{-/-}$ mice (Supplementary Figure S3D). ACE2 mRNA expression was increased in cPLA$_2$α$^{+/+}$ but not in cPLA$_2$α$^{-/-}$ mice (Supplementary Figure S3E).

DISCUSSION

This study demonstrates that cPLA$_2$α, that selectively releases AA from tissue lipids, is crucial for renal dysfunction, inflammation, and end-organ damage in Ang II-induced hypertension, most likely due to increased production and activity of predominantly pro-hypertensive eicosanoids and generation of ROS. In a previous study we showed that Ang II increased BP in cPLA$_2$α$^{+/+}$ but not cPLA$_2$α$^{-/-}$ mice.[20] The

effect of Ang II examined in the kidneys of these mice showed increased renal cPLA$_2$ activity without altering its expression in cPLA$_2$α$^{+/+}$ mice. This observation together with our previous demonstration that Ang II increases urinary output of AA metabolites, PGE2, TxB2, and 20-HETE in cPLA$_2$α$^{+/+}$ but not cPLA$_2$α$^{-/-}$ mice,[20] and that cPLA$_2$α gene disruption also inhibits the basal, and furosemide-induced increase in urinary PGE2 excretion,[3] suggest that eicosanoids generated by Ang II in the kidney are most likely due to AA release consequent to activation of cPLA$_2$α.

It has been shown that cPLA$_2$ gene disruption does not alter basal renal function; but it produces a concentration defect in older mice (>45 weeks).[3] In the present study, cPLA$_2$ gene disruption also did not alter basal renal
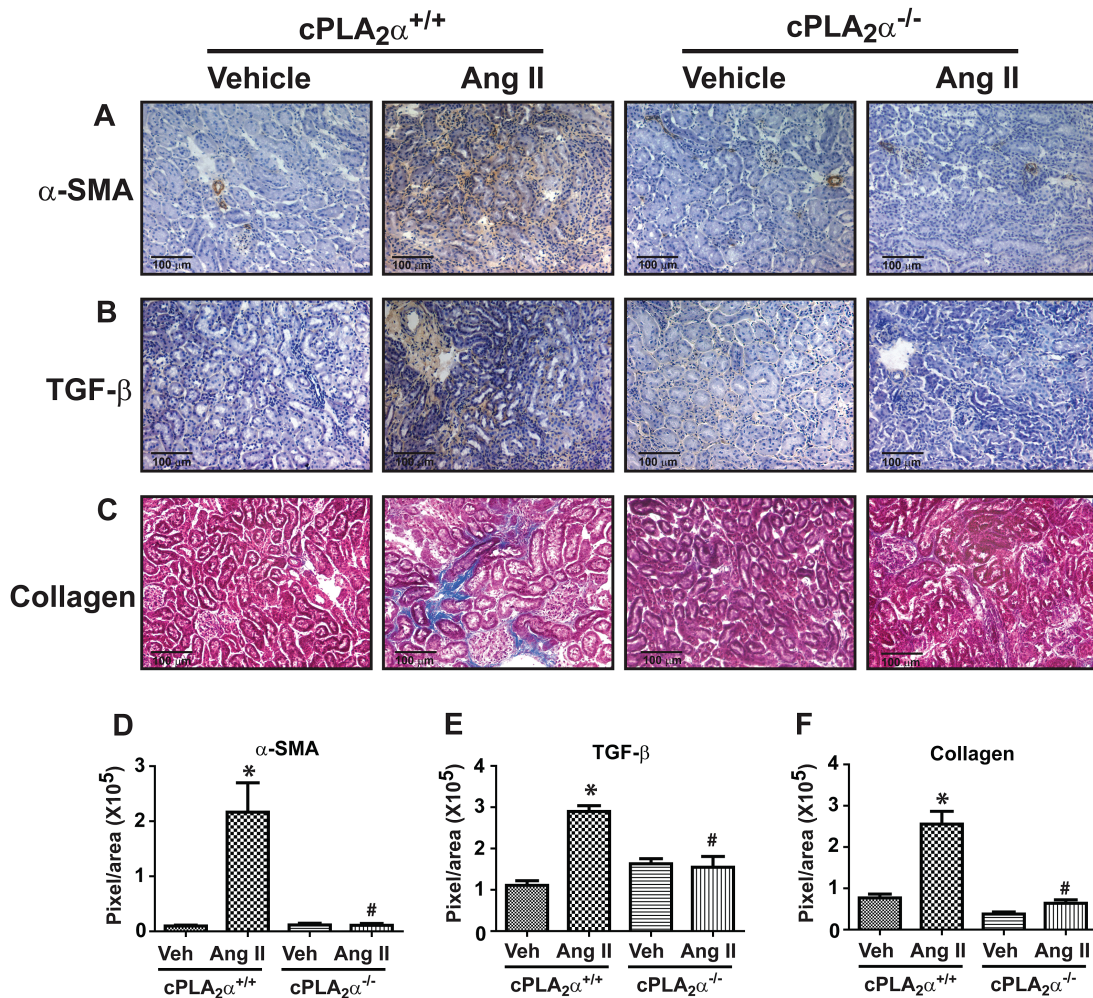
Figure 4. cPLA$_2$α gene disruption prevents renal fibrosis. Immunohistochemical analysis for α-smooth muscle actin (α-SMA) (**A**), transforming growth factor-β (**B**), and Masson's trichrome staining for collagen (**C**) in renal sections. Panels (**D**), (**E**), and (**F**) represent quantified data. *$P < 0.05$ cPLA$_2$α$^{+/+}$ vehicle vs. cPLA$_2$α$^{+/+}$ Ang II, #$P < 0.05$ cPLA$_2$α$^{+/+}$ Ang II vs. cPLA$_2$α$^{-/-}$ Ang II ($n = 3$–5 for each group, data are expressed as mean ± SEM).

function in younger mice. However, Ang II increased renal arterial resistance and pulsatility in cPLA$_2$α$^{+/+}$ but not in cPLA$_2$α$^{-/-}$ mice. This observation suggests that one or more AA metabolites, with pro-hypertensive effects, mediate Ang II-induced increase in renal vascular resistance in cPLA$_2$α$^{+/+}$ mice. Supporting this view, is the report that an inhibitor of AA metabolism, 5,8,11,14-eicosatetraynoic acid, attenuates Ang II-induced renal vasoconstriction.[23] PGE2 through EP1 and EP3 receptors[14] and PGH2 and TxA2 via Thromboxane receptor contribute to the pressor action of Ang II.[15,16] The demonstration that chronic blockade of thromboxane synthase attenuates Ang II-induced mesenteric artery vasosconstriction,[24] and disruption of Thromboxane receptor attenuates Ang II- induced hypertension and cardiac hypertrophy,[25] suggests that TxA2 is a significant component of pro-hypertensive eicosanoids that contribute to Ang II-induced hypertension. A recent study showed that PGE2 via activation of EP4 receptor increased the expression of (pro) renin receptor in rat renal medulla, and renin activity in medulla and urine, that partly contributes to Ang II-induced hypertension.[26] Products of AA

generated via lipoxygenase (12-HETE) and cytochrome P450 4A (20-HETE) also contribute to Ang II-induced renal vasoconstriction.[17,18,27] The increase in pulsatility and the resistive index in renal arteries correlates with long-term progression in chronic renal failure,[28] suggesting that pro-hypertensive products of AA contribute to renal dysfunction in Ang II-induced hypertension.

Ang II infusion for 13 days did not alter kidney: body weight ratio, food intake, or glomerular filtration rate as indicated by lack of change in plasma creatinine clearance but increased water intake, urine output, and Na+ excretion, and decreased urinary osmolality and caused proteinuria in cPLA$_2$α$^{+/+}$ mice. Each of these effects, however, were minimized in cPLA$_2$α$^{-/-}$ mice, suggesting that AA metabolites most likely contribute to these actions of Ang II. The cPLA$_2$α-dependent dipsogenic effect of Ang II could be mediated by the central actions of TxA2, because TxA2 receptor blockade inhibits while its activation enhances this effect of intracerebroventricularly administered Ang II.[29] Ang II stimulates the production of eicosanoids with both pro- and antihypertensive actions, and the
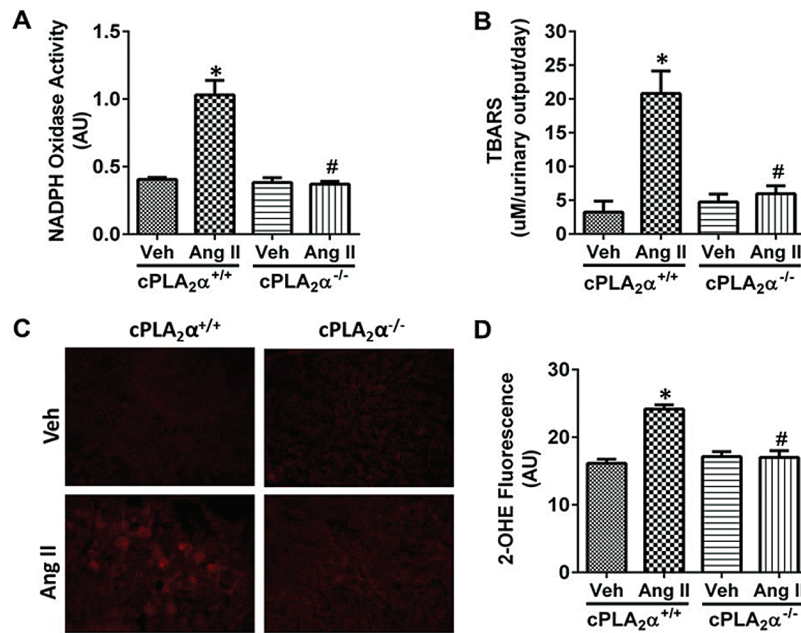
Figure 5. cPLA₂α gene disruption protects against angiotensin II (Ang II)-induced increase in renal oxidative stress. NADPH oxidase activity measured in kidney homogenate (**A**), urinary thiobarbituric acid reactive substances (TBARS) (**B**), ROS production determined by DHE staining (**C**) quantified as fluorescence of 2-OHE (**D**). *$P < 0.05$ cPLA₂α[+/+] vehicle vs. cPLA₂α[+/+] Ang II, #$P < 0.05$ cPLA₂α[+/+] Ang II vs. cPLA₂α[−/−] Ang II ($n = 6$ for each group, data are expressed as mean ± SEM).

balance between their vascular and tubular actions most likely maintains renal homeostasis which can become maladaptive with persistently higher levels of Ang II.[6,8–13,27,30] Therefore, it appears that renal dysfunction associated with Ang II-induced hypertension in cPLA₂α[+/+] mice, minimized in cPLA₂α[−/−] mice, is primarily mediated by pro-hypertensive eicosanoids. Urinary Na⁺ excretion was increased by Ang II in cPLA₂α[+/+] mice, most likely due to pressure diuresis. cPLA₂α gene disruption, however, prevented the effect of Ang II to increase urinary Na⁺ excretion, most likely due to decrease in BP and renal levels of natriuretic eicosanoids including PGE2, 12-HETE, 20-HETE, and 11,12-epoxyeicosatrienoic acid.[9–13] Further studies on the effect of various eicosanoids on renal function in cPLA₂α[−/−] mice would be required to address this issue. Since nonsteroidal anti-inflammatory COX-2 inhibitors or COX gene disruption produce renal dysfunction and hypertension in mice on a high salt diet due to loss of antihypertensive and renoprotective effect of PGI2,[30] studies in cPLA₂α[−/−] mice on low and high salt diets and other models of hypertension should allow further assessment of the role of eicosanoids with pro- and antihypertensive effects on renal function.

cPLA₂α is also present in immune cells. AA, released by its activation, is metabolized into eicosanoids with pro- and anti-inflammatory properties and is able to generate cytokines/chemokines, which regulate both innate and adaptive immune responses.[31,32] In our study, Ang II caused renal inflammation as demonstrated by increased F4/80⁺ macrophages and CD3⁺ T lymphocytes infiltration and damage as indicated by accumulation of α-smooth muscle actin⁺ myofibroblasts, transforming growth factor-β, and collagen in the kidneys of cPLA₂α[+/+] mice. The demonstration that

Ang II infusion failed to produce these effects in cPLA₂α[−/−] mice suggests that AA released by cPLA₂α activation results in production of eicosanoids that stimulate infiltration of macrophages and CD3⁺ T lymphocytes in the kidney resulting in inflammation and renal fibrosis.

The effect of cPLA₂α gene disruption to minimize Ang II-induced renal dysfunction, damage, and inflammation without altering basal renal function suggests that cPLA₂α activity, and the subsequent release of AA under basal physiological conditions, is low and does not appear to generate sufficient amount of eicosanoids to affect renal function. In the present study, Ang II increased renal cPLA₂ activity. Taken together with our recent finding that Ang II increases urinary excretion of AA metabolites in cPLA₂α[+/+] but not cPLA₂α[−/−] mice[20] suggests that the increased cPLA₂ activity observed in diabetic nephropathy,[33] glomerular nephritis,[34] and poly cystic kidney disease[35] is most likely due to associated increase in the activity of various vasoactive systems including the renin-angiotensin system.[36] Increased levels of Ang II would result in increased calcium influx in various renal cell types, AA release, and generation of eicosanoids that favor renal pro-hypertensive mechanisms and contribute to renal dysfunction, inflammation, and end-organ damage.

The mechanism by which cPLA₂α gene disruption minimizes Ang II-induced renal dysfunction, inflammation, and damage could be related in part to decreased BP.[37] The mechanical stretch and inflammation that are associated with hypertension promote aortic stiffening via activation of p38 mitogen-activated protein kinase.[38] The mechanical stretch increases Ca²⁺ influx via stress-operated Ca²⁺ channels, which is known to increase cPLA₂ activity and generation of eicosanoids,[39] and metabolites of AA increase p38

mitogen-activated protein kinase activity.[40] Therefore, it is possible that the effect of mechanical stretch caused by high BP on cardiovascular remodeling, activation of immune cells, and end-organ damage might be mediated in part by the effect of pro-hypertensive eicosanoids. However, Ang II also produces cardiovascular and renal pathophysiological changes independent of increased BP.[41] In transgenic rats carrying both human renin and angiotensinogen genes, treatment with triple therapy (hydralazine, reserpine, and hydrochlorothiazide) prevented increase in BP but not end-organ damage, inflammation, or cellular growth in the kidney.[41] Therefore, the protection against Ang II-induced renal dysfunction, inflammation, and damage could also result from a pressure-independent mechanism. Endothelin has been implicated in Ang II-induced hypertension and some of its renal actions.[21,22] However, in our study, endothelin does not appear to mediate $cPLA_2\alpha$-dependent actions of Ang II as its serum levels increased insignificantly to the same degree in both $cPLA_2\alpha^{+/+}$ and $cPLA_2\alpha^{-/-}$ mice.

The protective effect of $cPLA_2\alpha$ gene disruption against renal dysfunction, inflammation, and end-organ damage could be due to alterations by eicosanoids in expression of one or more Ang II receptors, Mas receptor, or ACE and ACE2. Ang II increased renal mRNA expression of $AT1_AR$ and AT2R and decreased ACE and increased ACE2 expression in $cPLA_2\alpha^{+/+}$ mice. Since these effects of Ang II were minimized in $cPLA_2\alpha^{-/-}$ mice, they are most likely mediated by eicosanoids. The increase in expression of $AT1_AR$ receptors could contribute to the renal effects of Ang II in $cPLA_2\alpha^{+/+}$ mice. The increase in mRNA expression of AT2R and ACE2 and decrease in ACE in $cPLA_2\alpha^{+/+}$ mice could be a compensatory mechanism activated by Ang II. Since expression of AT2R, MAS receptor and ACE2 were reduced in the kidneys of $cPLA_2\alpha^{-/-}$ compared to $cPLA_2\alpha^{+/+}$ mice, it appears that they are unlikely to contribute to reno-protection against the effects of Ang II in $cPLA_2\alpha^{-/-}$ mice.

Ang II is known to increase oxidative stress, activate immune cells that release cytokines, and promote inflammation that have been implicated in the development of hypertension and end-organ damage.[42,43] Our finding that $cPLA_2\alpha$ gene disruption prevented Ang II-induced increase in NADPH oxidase activity, generation of ROS and thiobarbituric acid reactive substances, suggests that ROS/lipid peroxides generated by renal and immune cells by AA and/or its metabolites most likely contribute to renal dysfunction, inflammation, and end-organ damage. Supporting this view are the reports that (i) monocytes/macrophages and T cells express $cPLA_2\alpha$[44] and NADPH oxidase,[45–47] (ii) AA is required for activation of NADPH oxidase and generation of ROS in monocyte/macrophages and vascular smooth muscle cells,[45,46,48] and (iii) ROS and/or one or more AA metabolites stimulate release of cytokines that promote inflammation.[49]

In conclusion, this study demonstrates that $cPLA_2\alpha$ is essential for the development of renal dysfunction, inflammation, and end-organ damage associated with Ang II-induced hypertension, most likely via generation of eicosanoids with pro-hypertensive effects and ROS/lipid peroxides. Therefore, $cPLA_2\alpha$ could serve as a potential novel target for developing therapeutic agents for treating renal dysfunction and end-organ damage associated with hypertension. Moreover, the development of water-soluble selective $cPLA_2\alpha$ inhibitors would allow further assessment of its physiological and pathophysiological significance in kidney diseases in other models of hypertension.

SUPPLEMENTARY MATERIAL

Supplementary materials are available at *American Journal of Hypertension* (http://ajh.oxfordjournals.org).

DISCLOSURE

The authors declared no conflict of interest.

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