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CYTOSOLIC PHOSPHOLIPASE A₂ α IS CRITICAL FOR ANGIOTENSIN II – INDUCED HYPERTENSION AND ASSOCIATED CARDIOVASCULAR PATHOPHYSIOLOGY

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Abstract

Angiotensin II activates cPLA₂ α and releases arachidonic acid from tissue phospholipids which mediate or modulate one or more cardiovascular effects of angiotensin II and has been implicated in hypertension. Since arachidonic acid release is the rate limiting step in eicosanoid production, cPLA₂ α might play a central role in the development of angiotensin II-induced hypertension. To test this hypothesis, we investigated the effect of angiotensin II infusion for 13 days by micro-osmotic pumps on systolic blood pressure and associated pathogenesis in wild type (cPLA₂ α ^{+/+}) and cPLA₂ α ^{-/-} mice. Angiotensin II-induced increase in systolic blood pressure in cPLA₂ α ^{+/+} mice was abolished in cPLA₂ α ^{-/-} mice; increased systolic blood pressure was also abolished by the arachidonic acid metabolism inhibitor, 5,8,11,14-eicosatetraynoic acid in cPLA₂ α ^{+/+} mice. Angiotensin II in cPLA₂ α ^{+/+} mice increased cardiac cPLA₂ activity and urinary eicosanoid excretion, decreased cardiac output, caused cardiovascular remodeling with endothelial dysfunction and increased vascular reactivity in cPLA₂ α ^{+/+} mice; these changes were diminished in cPLA₂ α ^{-/-} mice. Angiotensin II also increased cardiac infiltration of F4/80⁺ macrophages and CD3⁺ T lymphocytes, cardiovascular oxidative stress, expression of endoplasmic reticulum stress markers p58^{IPK} and CHOP in cPLA₂ α ^{+/+} but not cPLA₂ α ^{-/-} mice. Angiotensin II increased cardiac activity of ERK1/2 and cSrc in cPLA₂ α ^{+/+} but not cPLA₂ α ^{-/-} mice. These data suggest that angiotensin II-induced hypertension and associated cardiovascular pathophysiological changes are mediated by cPLA₂ α activation, most likely through the release of arachidonic acid and generation of eicosanoids with predominant pro-hypertensive effects and activation of one or more signaling molecules including ERK1/2 and cSrc.

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Conflicts of Interest/Disclosures

None.

Keywords

angiotensin II; cytosolic phospholipase A₂α (cPLA₂α); mice; cardiovascular function; fibrosis and hypertrophy; oxidative stress; inflammation

Introduction

The renin-angiotensin system (RAS) is a major component of the mechanisms that regulate cardiovascular and renal homeostasis, and an increase in its activity leads to the development of hypertension.¹ Angiotensin II (Ang II), the major bioactive peptide of the RAS, activates phospholipase A₂α (PLA₂α) and releases arachidonic acid (AA) from tissue phospholipids.² AA is metabolized by cyclooxygenase (COX) into thromboxane A₂ and prostaglandins (PG) E₂, F₂α, D₂, and I₂.³ Lipoxygenase (LO) converts AA into leukotrienes and 5-, 12- and 15-hydroxyeicosatrienoic acids (HETEs).⁴ AA is also metabolized by CYP4A via its ω-hydroxylase activity into 20-HETE, and via its epoxygenase activity into epoxyeicosatrienoic acids.^{5,6} These eicosanoids exert pro- or anti-hypertensive effects. PGE₂ via activation of EP₂ and EP₄ receptors,^{7,8} PGI₂,³ and epoxyeicosatrienoic acids,^{5,6,9,10} contribute to anti-hypertensive mechanisms whereas PGE₂ via activation of EP₁ and EP₃ receptors,⁸ and thromboxane A₂,³ 12- and 20-HETE^{6,10,11} by their vascular effects, contribute to pro-hypertensive mechanisms. AA metabolism has also been linked to the generation of reactive oxygen species (ROS)¹⁰ that have in turn been implicated in the development of hypertension.¹¹ The balance between pro- and anti-hypertensive eicosanoids, together with direct effects of various neurohumoral agents on the cardiovascular and renal systems, determines the level of blood pressure (BP). Whether AA metabolites generated via different pathways, following their release by activation of PLA₂ by Ang II, contribute primarily to pro- or anti-hypertensive mechanisms, is not known.

At least 16 groups of mammalian PLA₂ enzymes hydrolyze phospholipids to release fatty acids.¹² However, cPLA₂ selectively hydrolyzes AA containing phospholipids.¹² cPLA₂α has been implicated in a number of physiological and pathophysiological conditions¹³ including allergy,¹⁴ asthma,¹⁵ brain ischemia injury,¹⁶ collagen-induced arthritis^{17,18} and myocardial ischemia-reperfusion injury¹⁹. Several BP regulating hormones, including Ang II, activate cPLA₂ and release AA.^{20–23} Therefore the possibility arises that cPLA₂α, which is highly selective in releasing AA, might function as the major center of convergence for the transduction of hormone signaling to eicosanoid production and regulation of BP.

PLA₂ activity is increased in the renal medulla and cortex of stroke-prone spontaneously hypertensive rats²⁴ and a recent study showed the involvement of cPLA₂α in L-NG-nitroarginine methyl ester, an inhibitor of NO synthesis-induced hypertension.²⁵ However, the contribution of cPLA₂α to other forms of hypertension and more importantly its significance in cardiovascular remodeling and dysfunction, oxidative stress, and inflammation in Ang II-induced hypertension and the underlying mechanism, is not known and is the major objective of this study. These results show that cPLA₂α is crucial for the development of Ang II-induced hypertension and associated cardiovascular dysfunction,

oxidative stress, inflammation and remodeling, most likely due to the effect of predominantly pro-hypertensive eicosanoids generated from AA released by this lipase.

Methods

The detailed experimental methods are described in the online-only Data Supplement at <http://hyper.ahajournals.org>.

Results

cPLA₂ α contributes to the development of Ang II-induced hypertension and activation of cardiac cPLA₂ in mice

Ang II infusion over a period of 13 days, produced a consistent and reproducible increase in systolic BP (SBP), measured by the tail cuff (Figure 1A). Systolic (Figure 1B), diastolic (Figure 1C), and mean arterial BP (Figure 1D) measured by radio telemetry (Figure S1: tracing from all experiments), in cPLA₂ α ^{+/+} but not cPLA₂ α ^{-/-} mice. Basal BP was not different between these two phenotypes. Tracings of the BP measured by radio telemetry for each individual cPLA₂ α ^{+/+} and cPLA₂ α ^{-/-} mouse during Ang II infusion is shown in Figure S1–A1 and A-2. Heart rate during Ang II infusion as measured by radio telemetry was not altered in cPLA₂ α ^{+/+} or cPLA₂ α ^{-/-} mice (Figure S1B). Power spectral analysis of BP and heart rate variability showed an increase in the ratio of low frequency: high frequency heart rate variability during Ang II infusion in cPLA₂ α ^{+/+} mice indicating increased sympathetic outflow. This ratio was decreased in cPLA₂ α ^{-/-} mice (Figure S1C). Ang II also resulted in increased cPLA₂ activity measured by its phosphorylation, but not its protein expression, in cardiac tissue of Ang II infused cPLA₂ α ^{+/+} mice; the expression of cPLA₂ protein was absent in cPLA₂ α ^{-/-} mice (Figure S1D).

Arachidonic acid metabolism mediates Ang II induced hypertension in cPLA₂ α ^{+/+} mice

To elucidate the mechanism by which Ang II mediates hypertension in WT mice, another group of cPLA₂ α ^{+/+} mice were infused with Ang II and concurrently injected with an inhibitor of AA metabolism, 5,8,11,14 eicosatetraynoic acid (ETYA), at a dose of 50 mg/kg, i.p. every third day. ETYA prevented Ang II induced increase in SBP in cPLA₂ α ^{+/+} mice (Figure S2)

Ang II infusion resulted in a significant increase in excretion of PGE₂ metabolites (PGEM: 13,14-dihydro-15-keto PGA₂, 13,14-dihydro-15-keto PGE₂) (Figure 2A), TXB₂ (Figure 2B) and 20-HETE (Figure 2C), in cPLA₂ α ^{+/+} but not cPLA₂ α ^{-/-} mice.

cPLA₂ α gene disruption abolishes expression of cPLA₂ α but does not affect related phospholipases

The possibility arises that disrupting cPLA₂ α may alter the expression of other related phospholipases. Thus we performed q-RT PCR analysis for various phospholipases. mRNA expression of cPLA₂ α , but not other related enzymes, was absent in cardiac tissue of cPLA₂ α ^{-/-} mice (Figure S3A). Cardiac protein expression of phospholipase C β 2, D1, D2

was neither affected by cPLA₂ α disruption or Ang II infusion in either of the treatment groups (Figure S3B)

cPLA₂ α gene disruption does not alter expression of Ang II type 1 and 2, Mas receptor, and angiotensin converting enzyme

To determine if decreased BP during Ang II-infusion in cPLA₂ α ^{-/-} mice results from alterations in expression of Ang II type 1 (AT1) and 2 (AT2), Ang (1–7) Mas receptor, or angiotensin converting enzyme (ACE), we measured cardiac expression of these receptors and ACE. Expression of AT1, AT2, Mas receptor, and ACE were not altered in cardiac tissue of cPLA₂ α ^{-/-} mice (Figure S3C).

cPLA₂ α gene disruption prevents cardiac dysfunction and fibrosis associated with Ang II-induced hypertension in mice

Cardiac functional and structural changes were measured by echocardiography on the 12th day of infusion of Ang II or its vehicle. M-mode images of the LV (Left Ventricle) in the parasternal long-axis view showed dilated cardiomyopathy as indicated by LV chamber enlargement and impaired contractility in Ang II-infused cPLA₂ α ^{+/+} but not cPLA₂ α ^{-/-} mice (Figure 3A). Various parameters (Table 1), revealed cardiac dysfunction including an increase in LV mass in Ang II-infused cPLA₂ α ^{+/+} but not cPLA₂ α ^{-/-} mice. Heart sections of Ang II-infused cPLA₂ α ^{+/+} but not cPLA₂ α ^{-/-} mice showed fibrosis as indicated by positive staining of intracardiac α -smooth muscle actin (α -SMA) (Figure 3B), transforming growth factor (TGF)- β (Figure 3C), and collagen (Figure 3D).

cPLA₂ α gene disruption prevents cardiac inflammation

To determine the contribution of cPLA₂ α to inflammation associated with Ang II-induced hypertension, we examined the localization of F4/80⁺ macrophages, and CD3⁺ T-lymphocytes in cardiac tissue of cPLA₂ α ^{+/+} and cPLA₂ α ^{-/-} mice. Ang II caused infiltration of F4/80⁺ macrophages (Figure S4A) and CD3⁺ T cells (Figure S4B) in the hearts of cPLA₂ α ^{+/+} but not cPLA₂ α ^{-/-} mice.

cPLA₂ α gene disruption protects against Ang II-induced vascular remodeling

To assess the effect of cPLA₂ α gene disruption on vascular remodeling associated with Ang II-induced hypertension, aortas were stained with hematoxylin and eosin and media:lumen ratio was calculated. The aortas were also stained for α -SMA and collagen. Ang II increased media:lumen ratio (Figure 4A), α -SMA (Figure 4B) positive stained cells and collagen accumulation (Figure 4C) in aortas of cPLA₂ α ^{+/+} mice compared to cPLA₂ α ^{-/-} mice.

cPLA₂ α gene disruption prevents increased vascular reactivity and endothelial dysfunction in Ang II-induced hypertension

Ang II-induced hypertension was associated with increased response of aortas to phenylephrine (PE) (Figure S5A) and endothelin-1 (ET-1) (Figure S5B) in cPLA₂ α ^{+/+} mice; these increases were abrogated in cPLA₂ α ^{-/-} mice. Ang II-infusion caused endothelial dysfunction, as indicated by diminished dilation to acetylcholine (ACh) in aortas

precontracted with PE in cPLA₂α^{+/+} but not cPLA₂α^{-/-} mice (Figure S5C). Endothelial-independent relaxation of aorta to sodium nitroprusside (SNP) was not altered (Figure S5D).

cPLA₂α gene disruption protects against Ang II-induced cardiovascular oxidative stress

Oxidative stress has been implicated in various models of hypertension including Ang-II^{11,26}, and Ang II-induced activation of cardiac nicotinamide adenine dinucleotide phosphate (NADPH) oxidase depends on PLA₂ activity in vascular smooth muscle cells (VSMC) *in vitro*. Therefore, we investigated whether cPLA₂ gene disruption prevents Ang II-induced oxidative stress in heart and aorta. NADPH oxidase activity, measured by lucigenin-based luminescence assay, was increased in the hearts of Ang II-infused cPLA₂α^{+/+} but not cPLA₂α^{-/-} mice (Figure S6A). Infusion of Ang II increased cardiac (Figure S6B,C) and aortic (Figure S6D,E) ROS production in cPLA₂α^{+/+} but not in cPLA₂α^{-/-} mice measured by fluorescence of 2-hydroxyethidium generated after exposure of cardiac tissue and aorta to dihydroethidium.

cPLA₂α gene disruption prevents endoplasmic reticulum stress in Ang II-induced hypertension

Endoplasmic reticulum (ER) stress has been implicated in Ang II-induced hypertension.²⁷ To determine if Ang II-infusion promotes ER stress in the heart and if it is dependent on cPLA₂α, we measured mRNA expression of ER stress biomarkers p58^{IPK}, GRP78, XBP and CHOP (GADD153). Ang II infusion increased cardiac mRNA expression of p58^{IPK} that is induced in the early adaptive phase of the unfolded protein response, and CHOP (GADD153), a chronic ER stress marker²⁸ in cPLA₂α^{+/+} but not in cPLA₂α^{-/-} mice (Figure S7).

cPLA₂α gene disruption prevents Ang II-induced phosphorylation of extracellular signal-regulated kinase and cSrc

It is well established that, in VSMC, Ang II increases the production of ROS, and activity of one or more signaling molecules including extracellular signal-regulated kinase (ERK1/2) and cSrc that contributes to hypertrophy.²⁹ ERK1/2 also promotes phosphorylation of cPLA₂.³⁰ Ang II-infusion increased ERK1/2 (Figure S8A) and cSrc (Figure S8B) activity as measured by phosphorylation of these kinases in the heart tissue of cPLA₂α^{+/+} but not in cPLA₂α^{-/-} mice.

Discussion

The novel finding of this study is the demonstration that cPLA₂α is crucial for the development of Ang II-induced hypertension and associated cardiovascular dysfunction and hypertrophy, cardiac fibrosis, inflammation, oxidative stress, and activation of ERK1/2 and cSrc in mice. This conclusion is based on our finding that infusion of Ang II increased SBP, measured by tail cuff as well as radio telemetry, in cPLA₂α^{+/+} mice that was minimized by cPLA₂α gene disruption. The selectivity of this effect of cPLA₂α gene disruption in our mice was indicated by loss of cardiac expression of its mRNA but not that of other related Phospholipase enzymes. The protein expression of PLA₂ but not PLCβ2, PLD1, or PLD2 was also absent in cPLA₂α^{-/-} mice. Since cPLA₂α selectively catalyzes release of AA from

tissue lipids¹² and Ang II is known to activate cPLA₂ to release AA, cPLA₂ appears to mediate the hypertensive effect of Ang II via AA release. Supporting this view was our finding that cPLA₂ activity, as indicated by its phosphorylation, was increased in the heart of cPLA₂α^{+/+} but not cPLA₂α^{-/-} mice. The induction of eicosanoid production by lipopolysaccharide and calcium ionophore A23187 in peritoneal macrophages and furosemide-induced PGE₂ excretion was also abolished in cPLA₂α^{-/-} mice.^{16,31,32} In the present study, Ang II infusion increased the urinary output of PGEM, TXB₂, 20-HETEs in cPLA₂α^{+/+} but not cPLA₂α^{-/-} mice. Moreover, administration of inhibitor of AA metabolism, ETYA, blocked Ang II-induced increase in SBP in cPLA₂α^{+/+} mice. Therefore, it appears that metabolites of AA with pro-hypertensive effects contribute to the development of hypertension caused by Ang II in these mice. Since cPLA₂α gene disruption or ETYA did not alter basal BP, it appears that cPLA₂α activation and release of AA and its metabolites are not required to maintain basal BP.

The increase in BP produced by Ang II in cPLA₂α^{+/+} mice that was associated with cardiac dysfunction as indicated by decreased ejection fraction, fractional shortening, cardiac output and increased end diastolic volume and end systolic volume, cardiac hypertrophy as shown by increased LV mass, were minimized in cPLA₂α^{-/-} mice, suggesting an essential role of cPLA₂α^{+/+} in cardiac dysfunction and hypertrophy. Moreover, in the present study, cardiac fibrosis and inflammation as indicated by increased intracardiac staining of α-SMA myofibroblasts, TGF-β, as well as by increased infiltration of F4/80⁺ and CD3⁺ cells in Ang II-infused cPLA₂α^{+/+} mice were prevented in cPLA₂α^{-/-} mice. These findings suggest that AA metabolites with pro-hypertensive effects also mediate cardiac fibrosis and inflammation associated with Ang II-induced hypertension. cPLA₂α was also found to be critical for increased vascular remodeling and reactivity associated with Ang II-induced hypertension characterized by an increase in various parameters such as media:lumen ratio, α-SMA, deposition of collagen, as well as by increased contractile response of aorta to PE and ET-1 in cPLA₂α^{+/+} but not cPLA₂α^{-/-} mice. Although the effect of Ang II on cardiovascular remodeling has been shown to be independent of an increase in BP,^{33–36} we cannot exclude the possibility that the protection against Ang II-induced cardiovascular remodeling in cPLA₂α^{-/-} mice could also be due to decreased BP. The precise mechanism by which increase in BP causes cardiovascular remodeling is not known. Since stretch can increase cPLA₂ activity and eicosanoid production,³⁷ it raises the possibility that the increased stretch associated with hypertension might also result in cPLA₂α activation and generation of eicosanoids that contribute to Ang II-induced cardiovascular remodeling. Ang II-induced hypertension is also known to be associated with endothelial dysfunction.²⁶ Our finding that Ang II-induced endothelial dysfunction, as indicated by diminished relaxation of aorta to ACh but not to SNP, an agent that acts directly on VSMCs, occurs selectively in cPLA₂α^{+/+} but not cPLA₂α^{-/-} mice, suggests that cPLA₂α is essential for endothelial dysfunction associated with Ang II-induced hypertension. Moreover, endothelial dysfunction in larger vessels is dependent on NO.³⁸ Hypertension caused by the inhibitor of NO synthesis, L-NG-nitroarginine methyl ester (LNAME), which has been attributed to increased activity of RAS and sympathetic nervous systems,^{39,40} is also associated with endothelial dysfunction in the aorta. Both hypertension and endothelial dysfunction are prevented in cPLA₂α^{-/-} mice.²⁵

The mechanism by which cPLA₂α gene disruption protects against Ang II-induced hypertension and cardiovascular remodeling, inflammation, increased vascular reactivity, and endothelial dysfunction could be due to alterations in expression of Ang II, Ang (1–7) receptors, and ACE enzyme. However, this possibility appears to be unlikely because the expression levels of AT1, AT2, Mas receptor and ACE enzyme examined in the heart were not different in cPLA₂α^{-/-} compared to cPLA₂α^{+/+} mice. Oxidative stress and activation of immune system have been implicated in various models of hypertension including Ang II-induced hypertension.^{41,42} In the present study, Ang II-induced hypertension was associated with increased oxidative stress, as shown by increased cardiac NADPH oxidase activity and ROS production in the heart and aorta, and cardiac infiltration of F4/80⁺ macrophages and CD3⁺ T lymphocytes in cPLA₂α^{+/+} mice. These changes were prevented in cPLA₂α^{-/-} mice, suggesting that increased oxidative stress and inflammation in Ang II-induced hypertension are dependent on cPLA₂α. Supporting this view, it has been shown that Ang II increases NADPH oxidase activity by activating cPLA₂ and release of AA in VSMCs.^{43,44} cPLA₂α generated AA has also been implicated in NADPH oxidase activation in human monocytes and myeloid cell line PLB-985.^{45,46} AA metabolites generated by COX, PGE₂ via its actions on EP1 and EP3 receptors^{47,48} and TXA₂⁴⁹ contributes to Ang II-induced hypertension. AA metabolites formed by 12/15 lipoxygenase^{9,50} and by CYP450 4A (20-HETE),^{51,52} also contribute to Ang II-induced hypertension. Therefore, protection against Ang II-induced hypertension and associated cardiovascular pathogenesis in cPLA₂α^{-/-} mice is most likely due to lack of AA release and generation of one or more metabolites that mediate hypertensive effects of Ang II. Supporting this conclusion was our finding that Ang II infusion increased the urinary excretion of PGEM, TXB₂, 20-HETE in cPLA₂α^{+/+} but not cPLA₂α^{-/-} mice.

The site of pro-hypertensive eicosanoids generated by cPLA₂α that participate in Ang II-induced hypertension is not known. Since cPLA₂α is ubiquitously distributed in various tissues and eicosanoids generated from AA act locally, it is possible that eicosanoids generated at the site of action of Ang II including the cardiovascular, renal, brain and immune cells, could contribute to Ang II-induced hypertension. Recently, it has been shown that Ang II by stimulating expression of (pro)renin receptor in the rat renal medulla through COX₂-generated PGE₂, via stimulation of EP4 receptors increases renin release that partly contributes to Ang II-induced hypertension.⁵³ Also mice lacking macrophage 12/15 LO have been shown to be resistant to L-NAME or deoxycorticosterone-salt induced hypertension.⁵⁴ Ang II is known to cause hypertension by increasing oxidative stress in subfornical organ (SFO) of circumventricular organs and via its projections to paraventricular nucleus and from there to the brain stem, finally resulting in increased sympathetic activity.⁵⁵ cPLA₂α is also present in the brain^{13,16} and intracerebroventricular administration of PGE₂ increases sympathetic nervous activity, vasopressin release and BP.⁵⁶ The demonstration that the effect of Ang II in increasing BP is mediated by activation of the EP1 receptor by PGE₂, formed by COX-1 and not COX-2 in SFO, most likely by release of AA, suggests involvement of cPLA₂.⁵⁷ Therefore, it is possible that Ang II-induced hypertension in our study could be mediated by activation of cPLA₂α in the SFO as well as in the cardiovascular and renal systems. Moreover, it has been reported that Ang II-salt hypertension, which is associated with increased sympathetic activity and increased

plasma levels of norepinephrine, are minimized by inhibitor of COX-1, SC560 but not nemsulide, an inhibitor of COX 2 suggesting that COX-1 derived prostanoids by activating sympathetic nervous system, increase BP.⁵⁸ In contrast, COX2 inhibitors rofecoxib and nimesulide have been shown to attenuate Ang II-induced oxidative stress, hypertension and cardiac hypertrophy in rats.⁵⁹ The reason for the discrepancy between the two studies is unknown. Ang II is known to cause modulation of sympathetic outflow as determined by power spectral analysis of BP and heart rate variability⁶⁰. Our demonstration that the ratio of low frequency: high frequency heart rate variability as determined by power spectral analysis that was increased in cPLA₂ α ^{+/+} mice, was reduced in cPLA₂ α ^{-/-} mice, suggests that AA metabolites also contributes to modulation of sympathetic outflow by Ang II.

The oxidative stress produced by Ang II in SFO is mediated by ER stress, and inhibitors of ER stress minimize Ang II-induced hypertension.²⁷ Activation of cPLA₂ is associated with increased ER stress.⁶¹ Inhibitors of ER stress reduce BP in spontaneously hypertensive rats and the effect of endothelial-derived contractile factors, by suppressing H₂O₂ production and expression of COX-1, ERK1/2 and cPLA₂ phosphorylation.⁶² However, our demonstration that cPLA₂ α gene disruption prevented the cardiac expression of ER stress markers p58^{IPK} and CHOP suggests that Ang II also increases ER stress in the heart, but cPLA₂ α acts upstream of ER stress and NADPH oxidase activity, most likely by generating AA/metabolites. The effect of vasoactive agents, including Ang II, to promote influx of calcium and translocation of cPLA₂ α to the nuclear envelope/ER,⁶³ the site of AA metabolizing enzymes (COX, LO, and CYP P450),⁶⁴⁻⁶⁶ raises the possibility that cPLA₂ α via AA release and its metabolism by these enzymes might regulate generation of ER stress and ROS production. In support of this view, 12/15-LO has been implicated in ER stress in adipocytes, pancreatic islets, and rat liver.⁶⁵

The increased ROS generated by Ang II promotes cardiovascular remodeling by activating one or more signaling molecules.²⁹ Our finding that Ang II-induced hypertension was associated with increased cardiac ERK1/2 and cSrc activity in cPLA₂ α ^{+/+} but not in cPLA₂ α ^{-/-} mice suggests that these signaling molecules are most likely activated by oxidative stress produced by cPLA₂ α -generated AA metabolites, thus contributing to cardiovascular remodeling.

In conclusion, the present study provides the first evidence that the selective release of AA by cPLA₂ α is crucial for the development of Ang II-induced hypertension and associated cardiovascular pathophysiological changes including cardiovascular remodeling, increased vascular reactivity, endothelial dysfunction, and cardiac inflammation. These effects are most likely mediated by oxidative^{11,26,29} and ER stress^{27,62} generated by AA metabolism⁶⁵ and predominantly by pro-hypertensive eicosanoids resulting in activation of one or more signaling molecules including ERK1/2 and cSrc. However, studies using tissue specific knockout of cPLA₂ α and AA metabolizing enzymes would allow further assessment of their relative contribution in various tissues to Ang II- and other models of hypertension and associated pathogenesis.

Perspectives

The present study, using cPLA₂α^{+/+} and cPLA₂α^{-/-} mice, demonstrates that cPLA₂α selectively promotes release of AA from tissue phospholipids, a process that is critical for the development of Ang II-induced hypertension and associated cardiovascular remodeling and dysfunction, ER and oxidative stress, and inflammation. Moreover, our study suggests that cPLA₂α might function as the major center of convergence for the transduction of hormone signaling and that endogenously released AA results in the production of eicosanoids that predominantly favor pro-hypertensive mechanisms including oxidative and ER stress and inflammation. Therefore, the development of water-soluble selective inhibitors of cPLA₂α activity would allow assessing further, its contribution in cardiovascular and renal dysfunction in hypertension and its associated pathogenesis and treatment.

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Novelty and Significance

What is New?

- Evidence that cPLA₂α is essential for the development of Ang II-induced hypertension and associated cardiovascular remodeling, cardiac and endothelial dysfunction, and increased vascular reactivity.
- Cardiac oxidative and ER stress and inflammation associated with Ang II-induced hypertension also depends on cPLA₂α.
- Ang II-induced cardiac dysfunction, remodeling, and inflammation that depend on cPLA₂α are most likely mediated by ERK1/2 and cSrc.

What is Relevant?

- Our study provides novel information on the mechanism of Ang II-induced hypertension and associated cardiovascular pathophysiological changes, whereby cPLA₂α-generated AA and its metabolism to eicosanoids with pro-hypertensive effects is crucial for Ang II-induced hypertension.
- These findings suggest that development of agents that selectively inhibit cPLA₂α activity could be useful in treating hypertension and its pathogenesis.

Summary

The present study demonstrates that cPLA₂α gene disruption and AA metabolism inhibitor ETYA minimize Ang II-induced hypertension. Ang II-induced increase in SBP that was associated with increased urinary excretion of eicosanoids, cardiac dysfunction studied by echocardiography, cardiovascular remodeling, increased vascular reactivity, and endothelial dysfunction in cPLA₂α^{+/+} mice was markedly reduced in cPLA₂α^{-/-} mice. Infusion of Ang II also increased cardiac infiltration of inflammatory cells, NADPH oxidase activity, and cardiovascular oxidative stress and expression of ER stress markers in cPLA₂α^{+/+} but not cPLA₂α^{-/-} mice. Ang II increased cardiac ERK1/2 and cSrc activity in cPLA₂α^{+/+} but not cPLA₂α^{-/-} mice. We conclude that cPLA₂α is critical for Ang II-induced hypertension and associated cardiovascular dysfunction, remodeling, oxidative stress, and inflammation, most likely via AA release and generation of eicosanoids with pro-hypertensive effects and activation of ERK1/2 and cSrc.

Figure 1. cPLA₂α contributes to the development of Ang II-induced hypertension in mice cPLA₂α^{+/+} and cPLA₂α^{-/-} mice were infused with either Ang II (700 ng/kg/min) or its vehicle (0.9% saline) with micro-osmotic pumps for 13 days, and SBP was measured by tail cuff (A) and radio telemetry (B). Diastolic (C) and mean arterial pressure (D) were also measured by telemetry **P* < 0.05 cPLA₂α^{+/+} vehicle vs. cPLA₂α^{+/+} Ang II, #*P* < 0.05 cPLA₂α^{+/+} Ang II vs. cPLA₂α^{-/-} Ang II (n = 4–6 for all experiments, and data are expressed as mean ± SEM, data was analyzed by RM two-way analysis of variance followed by Turkey's multiple comparison tests)

Figure 2. Increased arachidonic acid metabolism to pro-hypertensive eicosanoids mediates Ang II induced hypertension in cPLA₂α^{+/+} mice

Increase urinary excretion of PGE2 metabolites (**A**) TXB2 (**B**) and 20-HETE (**C**) was observed in cPLA₂α^{+/+} but not in cPLA₂α^{-/-} mice infused with Ang II. **P* < 0.05 cPLA₂α^{+/+} vehicle vs. cPLA₂α^{+/+} Ang II, #*P* < 0.05 cPLA₂α^{+/+} Ang II vs. cPLA₂α^{-/-} Ang II, ^φ *P* < 0.05 cPLA₂α^{+/+} vehicle vs. cPLA₂α^{-/-} vehicle (n = 4–6 for all experiments, and data are expressed as mean ± SEM)

Figure 3. cPLA₂α gene disruption attenuates cardiac dysfunction, hypertrophy and fibrosis associated with Ang II-induced hypertension in mice

Echocardiograms of the left ventricle in M mode, parasternal long-axis view (A), demonstrates increase in LVID in both systole (red) and diastole (green) suggesting dilated cardiomyopathy characterized by enlarged LV mass in Ang II-infused cPLA₂α^{+/+} but not cPLA₂α^{-/-} mice. Various parameters of cardiac function were also measured (Table 1). Increased interstitial staining of α-SMA (B) and TGF-β (C) indicators of interstitial fibrosis and collagen accumulation (D) (intense blue staining) was observed in Ang II-infused cPLA₂α^{+/+} but not cPLA₂α^{-/-} mice. (n=4–6 for each group; data are expressed as mean ± SEM)

Figure 4. cPLA₂α gene disruption protects against Ang II-induced vascular remodeling
 Sections of aortas were stained and quantified for media:lumen ratio (A) α-smooth muscle actin (B), and collagen accumulation (C). **P* < 0.05 vehicle vs. Ang II, #*P* < 0.05 cPLA₂α^{+/+}Ang II vs. cPLA₂α^{-/-}Ang II. (n=3–5 for each group; data are expressed as mean ± SEM)

Table 1

Parameters of Cardiac Function

Parameters	cPLA ₂ α ^{+/+}		cPLA ₂ α ^{-/-}	
	Vehicle	Ang II	Vehicle	Ang II
EF%	63.94 ± 1.0	40.66 ± 3.2 [*]	63.47 ± 2.4	63.97 ± 1.3 [#]
FS%	34.19 ± 0.8	19.39 ± 1.9 [*]	33.77 ± 1.7	34.17 ± 0.9 [#]
SV	39.78 ± 1.2	23.48 ± 1.6 [*]	37.33 ± 2.8	36.60 ± 2.5 [#]
CO	16.35 ± 0.6	11.80 ± 1.1 [*]	15.11 ± 0.7	16.84 ± 2.5 [#]
LV mass	83.07 ± 7.3	146.66 ± 6.2 [*]	92.90 ± 2.9	107.56 ± 7.8 ^{*#}
LVID-d	3.61 ± 0.2	4.31 ± 0.05 [*]	3.60 ± 0.2	3.66 ± 0.1 [#]
LVID-s	2.33 ± 0.1	3.63 ± 0.1 [*]	2.33 ± 0.2	2.37 ± 0.1 [#]
LVAW-s	1.24 ± 0.1	1.44 ± 0.2	1.31 ± 0.1	1.55 ± 0.1
LVAW-d	1.10 ± 0.1	1.36 ± 0.1	0.97 ± 0.1	1.07 ± 0.1
LVPW-s	0.95 ± 0.1	1.15 ± 0.01	1.22 ± 0.2	1.56 ± 0.1
LVPW-d	0.68 ± 0.03	1.05 ± 0.04	0.85 ± 0.1	0.91 ± 0.04
EDV	62.36 ± 1.8	75.78 ± 1.9 [*]	57.63 ± 4.7	58.59 ± 4.3 [#]
ESV	21.39 ± 0.7	53.50 ± 3.5 [*]	22.38 ± 2.9	21.97 ± 2.5 [#]
IVS-s	1.48 ± 0.02	1.44 ± 0.01	1.21 ± 0.1	1.65 ± 0.1
IVS-d	0.98 ± 0.04	1.06 ± 0.02	0.86 ± 0.1	1.05 ± 0.1

* EF, ejection fraction; FS, fractional shortening; SV, stroke volume (ul); CO, cardiac output (ml/min); LV mass, left ventricular mass (mgs); LVID, LV internal dimension (mm); LVAW, LV anterior wall (mm); LVPW, LV posterior wall (mm); EDV, end diastolic volume (ul); ESV, End systolic volume; IVS, Intraventricular septum (mm). s, d indicate systole or diastole. The parameters listed in the table were measured using M-mode and B-mode images in the short and parasternal long-axis views as described in the methods.

* $P < 0.05$ cPLA₂α^{+/+} vehicle vs. cPLA₂α^{+/+} Ang II,

[#] $P < 0.05$ cPLA₂α^{+/+} Ang II vs. cPLA₂α^{-/-} Ang II (n=4–6 for each group; data are expressed as mean ± SEM).