Brain Cytosolic Phospholipase A$_2$α Mediates Angiotensin II-Induced Hypertension and Reactive Oxygen Species Production in Male Mice

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BACKGROUND
Recently, we reported that angiotensin II (Ang II)-induced hypertension is mediated by group IV cytosolic phospholipase A$_2$α (cPLA$_2$α) via production of prohypertensive eicosanoids. Since Ang II increases blood pressure (BP) via its action in the subfornical organ (SFO), it led us to investigate the expression and possible contribution of cPLA$_2$α to oxidative stress and development of hypertension in this brain area.

METHODS
Adenovirus (Ad)-green fluorescence protein (GFP) cPLA$_2$α short hairpin (sh) RNA (Ad-cPLA$_2$α shRNA) and its control Ad-scrambled shRNA (Ad-Scr shRNA) or Ad-enhanced cyan fluorescence protein cPLA$_2$α DNA (Ad-cPLA$_2$α DNA) and its control Ad-GFP DNA were transduced into SFO of cPLA$_2$α$^{+/+}$ and cPLA$_2$α$^{-/-}$ male mice, respectively. Ang II (700 ng/kg/min) was infused for 14 days in these mice, and BP was measured by tail-cuff and radio telemetry. cPLA$_2$α activity, reactive oxygen species production, and endoplasmic reticulum stress were measured in the SFO.

RESULTS
Transduction of SFO with Ad-cPLA$_2$α shRNA, but not Ad-Scr shRNA in cPLA$_2$α$^{+/+}$ mice, minimized expression of cPLA$_2$α, Ang II-induced cPLA$_2$α activity and oxidative stress in the SFO, BP, and cardiac and renal fibrosis. In contrast, Ad-cPLA$_2$α DNA, but not its control Ad-GFP DNA in cPLA$_2$α$^{-/-}$ mice, restored the expression of cPLA$_2$α, and Ang II-induced increase in cPLA$_2$α activity and oxidative stress in the SFO, BP, cardiac, and renal fibrosis.

CONCLUSIONS
These data suggest that cPLA$_2$α in the SFO is crucial in mediating Ang II-induced hypertension and associated pathogenesis. Therefore, development of selective cPLA$_2$α inhibitors could be useful in treating hypertension and its pathogenesis.

Keywords: angiotensin II; blood pressure; cytosolic phospholipase A$_2$α; hypertension; cPLA$_2$α$^{+/+}$; cPLA$_2$α$^{-/-}$; cPLA$_2$α$^{+/−}$ mice; subfornical organ.

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Angiotensin (Ang) II, the main component of the renin–angiotensin system, plays an important role in the pathogenesis of cardiovascular diseases including hypertension.¹ Ang II-induced hypertension is due to its action in the subfornical organ (SFO) of circumventricular organs resulting in increased oxidative and endoplasmic reticulum (ER) stress and activity of the sympathetic nervous system.²⁻⁴ There is a substantial body of evidence that increased reactive oxygen species (ROS) production and activation of immune cells mediate Ang II-induced hypertension and associated pathogenesis.⁵⁻⁸ Ang II also increases the activity of cytosolic phospholipase A$_2$ (cPLA$_2$) resulting in arachidonic acid (AA) release from tissue phospholipids.⁹¹⁰ AA is metabolized by cyclooxygenase (COX), lipoxygenase, and cytochrome P450 into various eicosanoids with prohypertensive and antihypertensive effects.¹¹⁻¹³ Prostaglandin (PG) E₂, by stimulating EP1 and EP3 receptors,¹⁴ and thromboxane A₂,¹¹ 12-, and 20-HETE,¹₂,¹₃,¹₅,¹₆ by their vascular actions, exert prohypertensive effects. On the other hand, PGE₂ through stimulation of EP2 and EP4 receptors,¹⁴,¹⁷ PGI₂,¹¹ and epoxyeicosatrienoic acids¹⁸ produce vasodepressor effects. One or more of the eicosanoids contribute to Ang II-induced hypertension.¹⁹⁻²² Ang II-salt hypertension is also dependent on COX-1 activity.²³ Intracerebroventricular...
administration of PGE2 increases sympathetic activity, vaso-
pressin release, and blood pressure (BP).24 and the hypotha-
lamic paraventricular excitation and sympathetic activation,
via EP3 receptors.25 Injection of PGE2 into the rostral ven-
troterminal medulla also causes sympathoexcitation and pres-
sor response via the EP3 receptor.26 These observations
suggest that the release of AA by cPLA2, the rate-limiting
step in the synthesis of eicosanoids, could be critical for Ang
II-induced ROS production and hypertension.

Several types of mammalian cPLA2 enzymes have been
identified;27 however, group IV cPLA2 shows high selectivity
for AA-containing phospholipids.27,28 cPLA2 consists of six
isoforms (cPLA2α, -β, -γ, -δ, -ε, and -ζ) with only 30% homol-
y, tissue distribution, and enzymatic activity.28 In a previous
study, we showed that the selective cPLA2α gene disruption
prevented Ang II-induced increase in urinary levels of eico-
sanoids, hypertension, and associated cardiovascular, renal
dysfunction and inflammation, suggesting that prohyper-
tensive eicosanoids generated from AA mediate Ang II-induced
hypertension.29,30 However, the site of eicosanoids produced by
group IV cPLA2α, which mediate Ang II-induced hyperten-
sion, is not known. Since numerous tissues including cardio-
vascular, renal, brain, and immune cells produce eicosanoids
that exert their effect locally, these should be formed from AA
released by cPLA2α and act at the site of action of Ang II.

PLA2α is distributed in several regions of the brain,31 and Ang
II increases expression of PLA2α in the organum vasculosum
of the lamina terminalis, paraventricular nucleus (PVN), nucleus
of the solitary tract, and middle cerebral artery.32 The demon-
straation that Ang II-induced oxidative stress and hypertension
is mediated via the COX-1-derived metabolite PGE2 via EP1
receptor in the SFO33 raises the possibility that cPLA2α in the
SFO might be critical for the action of Ang II to increase ox-
idative stress and BP. To test this hypothesis, we examined
the localization and the effect of cPLA2α depletion in the SFO by
transduction with adenovirus (Ad)-green fluorescence protein
(GFP)-cPLA2α short hairpin (sh) RNA (Ad-cPLA2α shRNA).
We also examined its reconstitution in knockout (cPLA2α−/−)
mice by transduction with Ad-encoding human fluorescent
protein (ECFP)-cPLA2α DNA (Ad-cPLA2α DNA) in the
SFO. We then examined the effect of these probes on Ang
II-induced hypertension and associated pathogenesis in mice.
Our results show that depletion of cPLA2α in the SFO
prevents Ang II-induced hypertension, ROS and ER stress, and
associated pathogenesis, while expression of cPLA2α in cPLA2α−/−
mice restores these deleterious effects of Ang II.

MATERIALS AND METHODS

Details for Materials and Methods section are in the
online-only Data Supplement.

Animal experiments

All animal experiments were performed using protocols
approved by the University of Tennessee Health Science
Center Institutional Animal Care and Use Committee
according to the National Institutes of Health Guide for the
Care and Use of Laboratory Animals. Experiments were
conducted in 8- to 10-week-old, 20- to 25-g body weight,
wild-type (cPLA2α+/+), and cPLA2α gene disrupted homo-
zygous (cPLA2α−/−) male mice on BALB/c background.
Ang II (700 ng/kg/min) or saline (vehicle) was infused for
14 days with micro-osmotic pumps implanted subcutane-
ously. Systolic BP (SBP) was measured by the noninvasive
tail-cuff method, or mean arterial pressure (MAP) daily by
radio telemetry. However, 2 to 3 out of 6 cPLA2α+/− BALB/c
mice implanted with radio transmitters did not survive
more than 8 to 10 days. We did not encounter this problem
in male C57BL/6 mice. Therefore, we first confirmed the BP
measurements recorded by the tail-cuff method in the male
C57BL/6 mice to that obtained in BALB/c mice and then
used cPLA2α−/− mice on the C57BL/6 background to further
confirm BP measurements by radio telemetry.

Statistical analysis

One or 2-way analysis of variance was used to analyze the
data, Tukey’s post hoc test for multiple comparisons, and
student’s t-test to compare the difference between 2 groups.
The values obtained from at least 3 to 5 different experiments
were expressed as the mean ± SEM, P <0.05 was considered
statistically significant.

RESULTS

cPLA2α gene disruption in SFO of cPLA2α+/+ mice with
Ad-cPLA2α shRNA attenuated Ang II-induced increase in
BP and cPLA2α activity, but not expression of cPLA2α, and
reduced collagen accumulation in the heart and kidney

To determine the contribution of cPLA2α in the SFO to Ang
II-induced hypertension, the SFO was transduced with Ad-cPLA2α shRNA. Infusion of Ang II by micro-osmotic
pumps implanted subcutaneously increased SBP, measured by
tail-cuff, in male cPLA2α+/+ BALB/c and cPLA2α−/− C57BL/6
mice (Supplementary Figure S1A and B). Transduction of the
SFO with Ad-cPLA2α shRNA but not its Ad-Scr shRNA
prevented Ang II-induced increase in SBP in male cPLA2α+/−
BALB/c mice (Figure 1a). SBP was not altered by the adenovi-
ruses during vehicle infusion (Figure 1a). Ad-cPLA2α shRNA
but not its Ad-Scr shRNA also prevented Ang II-induced
increase in mean arterial BP (MAP) measured by radio telem-
etry in cPLA2α−/− C57BL/6 mice (Figure 1b). Transduction of the
SFO with Ad probes was confirmed by expression of GFP in
the SFO (Supplementary Figure S2A). cPLA2α expression
was abolished by Ad-cPLA2α shRNA but not Ad-Scr
shRNA as determined by cPLA2α immunoreactivity using
mouse anti-cPLA2α antibody in BALB/c mice (Figure 1c and
d), and by RT-PCR in BALB/c and C57BL/6 mice (Supplementary
Figures S2B and S3A, respectively). Ang II also increased cPLA2α
activity measured by increased phospho-cPLA2α immunoreac-
tivity in the SFO transduced with Ad-Scr shRNA, but not with
Ad-cPLA2α shRNA in BALB/c mice (Figure 1c and d), and
C57BL/6 mice (Supplementary Figure S3B and C). Transduction of the
SFO with Ad-Scr shRNA or Ad-cPLA2α shRNA did not alter
expression of cPLA2α in the PVN, heart, and kidney examined
in BALB/c mice (Supplementary Figure S2C–E).

Ang II is known to cause cardiac and renal fibrosis.29,30
To determine if the alteration in cPLA2α expression in the

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SFO also affects the action of Ang II on cardiac and renal fibrosis, we examined the accumulation of collagen in these tissues in BALB/c mice. cPLA\(_2\)\(^\alpha\) gene disruption in the SFO of cPLA\(_2\)\(^\alpha\)\(+/+\) mice by transduction with Ad-cPLA\(_2\)\(^\alpha\) shRNA but not its Ad-Scr shRNA control infused with Ang II minimizes accumulation of collagen in the heart and kidney (Supplementary Figure S4A and B).

**Transduction with Ad-ECFP-cPLA\(_2\)\(^\alpha\) DNA, but not Ad-GFP DNA in the SFO of cPLA\(_2\)\(^\alpha\)\(^{-/-}\) BALB/c mice restored the effect of Ang II to increase BP**

Ang II failed to increase BP in cPLA\(_2\)\(^\alpha\)\(^{-/-}\) BALB/c mice (Supplementary Figure S1). Transduction with Ad-cPLA\(_2\)\(^\alpha\) DNA but not Ad-GFP DNA in the SFO of cPLA\(_2\)\(^\alpha\)\(^{-/-}\) BALB/c mice restored the effect of Ang II to increase BP measured by tail-cuff (Figure 2a). Ang II also increased MAP measured by radio telemetry in cPLA\(_2\)\(^\alpha\)\(^{-/-}\) C57BL/6 mice transduced with Ad-cPLA\(_2\)\(^\alpha\) DNA, but not Ad-GFP DNA in the SFO (Figure 2b). The localization of ECFP-cPLA\(_2\)\(^\alpha\) and GFP in the SFO transduced with Ad-cPLA\(_2\)\(^\alpha\) DNA and Ad-GFP DNA, respectively, was confirmed by their fluorescence (Supplementary Figure S5A), and by RT-PCR in BALB/c (Supplementary Figure S5B) and C57BL/6 mice (Supplementary Figures S5A and S6A), and by immunohistochemistry using anti-cPLA\(_2\) antibody in BALB/c (Figure 2c and d) and C57BL/6 (Supplementary Figure S6B and C) mice. Ang II did not alter expression of

![Image of Figure 1](https://academic.oup.com/ajh/article-abstract/31/5/622/4802430)

**Figure 1.** cPLA\(_2\)\(^\alpha\) gene disruption in subfornical organ (SFO) of cPLA\(_2\)\(^\alpha\)\(^{-/-}\) mice with adenovirus (Ad)-green fluorescence protein (GFP)-cPLA\(_2\)\(^\alpha\) shirt hairpin (sh) RNA (Ad-cPLA\(_2\)\(^\alpha\) shRNA) abrogates Ang II-induced increase in blood pressure (BP) and cPLA\(_2\)\(^\alpha\) phosphoimmunoreactivity. Ad-GFP scramble (Scr) shRNA (Ad-Scr shRNA) or Ad-cPLA\(_2\)\(^\alpha\) shRNA was transduced into SFO. (a) systolic blood pressure (SBP) was measured by tail-cuff in BALB/c mice. (b) Mean arterial blood pressure (MAP) was measured by radio telemetry in C57BL/6 mice. (c) Expression of cPLA\(_2\)\(^\alpha\) and its activity measured by its phosphorylation in SFO of BALB/c mice by immunohistochemical method. Scale bars: 50 µm. (d) Quantified data. Data are expressed as mean ± SEM. n = 5 per group. *, **P < 0.05, Ad-Scr shRNA-Ang II vs. Ad-Scr shRNA-Veh (Vehicle); †P < 0.05, Ad-cPLA\(_2\)\(^\alpha\) shRNA-Ang II vs. Ad-Scr shRNA-Ang II in cPLA\(_2\)\(^\alpha\)\(+/+\) BALB/c mice (a) and cPLA\(_2\)\(^\alpha\)\(^{-/-}\) C57BL/6 mice (b). ‡P < 0.05, Ad-cPLA\(_2\)\(^\alpha\) shRNA vs. Ad-Scr shRNA.
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cPLA2α, but it increased the cPLA2 activity, measured by phospho-cPLA2α immunoreactivity in the SFO transduced with Ad-cPLA2α DNA, but not Ad-GFP DNA in BALB/c mice (Figure 2c and d). cPLA2α mRNA expression in the PVN, heart, and kidney that was absent in cPLA2α−/− BALB/c, was not altered by transduction of the SFO with Ad-GFP DNA or Ad-cPLA2α DNA during infusion of Ang II (Supplementary Figure S5C–E).

We also determined the effect of Ang II on collagen accumulation in the heart and kidney of BALB/c cPLA2α−/− mice transduced with Ad-cPLA2α DNA and Ad-GFP DNA in the SFO, and found collagen accumulation in the former but not the latter group of mice (Supplementary Figure S7A and B).

Transduction of SFO with cPLA2α shRNA in cPLA2α+/− mice attenuated, and Ad-cPLA2α DNA in cPLA2α−/− mice restored Ang II-induced increase in ROS production

These studies were conducted in BALB/c mice that were infused with Ang II or its vehicle for the measurement of BP as described above. Infusion of Ang II also stimulated the production of ROS as indicated by enhanced 2-hydroxyethidium fluorescence in the SFO generated after staining with dihydroethidium as described,4 in cPLA2α+/− mice but not in cPLA2α−/− mice (Figure 3a and b). Transduction of the SFO with Ad-cPLA2α shRNA but not its Ad-Scr shRNA inhibited dihydroethidium staining (Figure 3c and d) in cPLA2α+/− mice. Infusion of Ang II in cPLA2α−/− mice failed

Figure 2. cPLA2α gene transduction in subfornical organ (SFO) of cPLA2α−/− mice with adenovirus (Ad)-enhanced cyan fluorescence protein (ECFP)-cPLA2α DNA (Ad-cPLA2α DNA) restores Ang II-induced increase in blood pressure (BP) and cPLA2 activity. Ad-green fluorescence protein (GFP) (Ad-GFP) DNA or Ad-cPLA2α DNA was transduced into SFO. (a) Systolic blood pressure (SBP) was measured by tail-cuff in cPLA2α−/− BALB/c mice. (b) Mean arterial blood pressure (MAP) was measured by radiotelemetry in cPLA2α−/− C57BL/6 mice. (c) Expression, and activation of cPLA2 in SFO of BALB/c mice were measured by immunohistochemical method. Scale bars: 50 µm. (d) Quantified data. Data are expressed as mean ± SEM. n = 5 per group. *, ** P < 0.05, Ad-cPLA2α DNA-Ang II vs. Ad-cPLA2α DNA-Veh (vehicle); †P < 0.05, Ad-cPLA2α DNA-Ang II vs. Ad-GFP DNA-Ang II in cPLA2α−/− BALB/c and C57BL/6 mice. *P < 0.05, Ad-cPLA2α DNA vs. Ad-GFP DNA.
to increase dihydroethidium staining, whereas transduction with Ad-cPLA,α DNA, but not Ad-GFP DNA in the SFO of these mice restored the effect of Ang II to increase dihydroethidium staining (Figure 3e and f).

**Ang II increased ER stress marker expression in SFO Of cPLA,α+/− but not cPLA,α−/− mice**

Ang II also increased ER stress as indicated by increased mRNA levels of markers of ER stress glucose-related protein 78 (GRP78), and C/EBP homologous protein (CHOP) in cPLA,α−/− BALB/c mice (Figure 4a). Infusion of Ang II in cPLA,α−/− mice did not induce mRNA levels of GRP78 and CHOP (Figure 4b).

**Partial cPLA,α gene disruption (cPLA,α+/−) also prevented Ang-II-induced increase in BP in mice**

Ang II did not increase BP in cPLA,α−/− BALB/c and C57BL/6 male mice (Supplementary Figure S1A and B). To determine if partial cPLA,α gene disruption reduces Ang II-induced increase in BP, we examined its effect in heterozygous cPLA,α (cPLA,α+/−) C57BL/6 male mice. Ang II (700 ng/kg/min) increased SBP measured by tail-cuff in cPLA,α+/− but not cPLA,α−/− C57BL/6 male mice (Supplementary Figure S8). cPLA,α mRNA expression in the SFO, heart, and kidney of C57BL/6 cPLA,α+/− mice was lower (60–80%) than in C57BL/6 cPLA,α−/− mice (Supplementary Figure S9).

Figure 3. Transduction of subfornical organ (SFO) with Ad-cPLA,α shRNA attenuates, and Ad-cPLA,α DNA restores Ang II-induced increase in reactive oxygen species (ROS) production in cPLA,α+/− BALB/c mice. ROS production was determined using dihydroethidium (DHE) (a) and (b) Ang II-induced increase in ROS production in SFO in cPLA,α+/− but not cPLA,α−/− BALB/c mice. (c) and (d) Transduction of Ad-cPLA,α shRNA in SFO abrogated Ang II-induced increase in ROS production in cPLA,α+/− BALB/c mice. (e) and (f) Transduction of Ad-cPLA,α DNA in SFO restored Ang II-induced increase in ROS production in cPLA,α+/− BALB/c mice. Panels a, c, and e, scale bars: 50 μm. Panels b, d, and f, Quantified data (A. U., arbitrary units). Data are expressed as mean ± SEM. n = 5 per group. *P < 0.05, Ang II vs. Veh (vehicle); %P < 0.05, cPLA,α+/−-Ang II vs. cPLA,α+/−-Ang II; **P < 0.05, Ad-Scr shRNA-Ang II vs. Ad-Scr shRNA-Veh; †P < 0.05, Ad-cPLA,α shRNA-Ang II vs. Ad-Scr shRNA-Ang II; ‡P < 0.05, Ad-cPLA,α shRNA-Ang II vs. Ad-Scr shRNA-Ang II. (f) Transduction of Ad-Scr shRNA in SFO restored Ang II-induced increase in ROS production in cPLA,α+/− BALB/c mice. (Supplementary Figure S1A and B). Infusion of Ang II in cPLA,α+/− mice also prevented ROS production and ER stress (Figure 4a) and (Figure 4b).

**DISCUSSION**

The major findings of this study are that SFO is the principal site of action of cPLA,α in mediating the action of Ang II: (i) to increase BP; (ii) to stimulate ROS production and ER stress in the SFO, and (iii) to cause cardiac and renal fibrosis. These findings are based on our demonstration that cPLA,α selectively releases AA from tissue phospholipids, and is expressed in the SFO, and that Ang II increased cPLA,α activity, as determined by its phosphoimmunoreactivity without altering its expression. However, Ang II has been shown to increase expression of phospholipase A2 in the organum vasculosum of the lamina terminalis, PVN, nucleus of the solitary tract, and middle cerebral artery of the rat. Whether this increase in phospholipase A2 expression by Ang II in these tissues represents primarily increased expression of cPLA,α, or other isoforms of phospholipase A2 is not known. We have previously reported that Ang II increases BP, and sympathetic outflow as determined from heart rate variability by power spectral analysis in cPLA,α−/− but not cPLA,α+/− BALB/c mice. Our demonstration that cPLA,α gene disruption in the SFO by Ad-cPLA,α shRNA, but not its Ad-Scr shRNA, reduced cPLA,α expression and phospho-cPLA,α immunoreactivity and prevented Ang II-induced increase in BP in cPLA,α+/− BALB/c and C57BL/6 mice, suggests that cPLA,α in the SFO is critical for Ang II-induced hypertension. Although Ang II 700 ng/kg/min used in this study would be expected to cause the increase in BP by its direct vascular action but it appears that cPLA,α in the SFO is primarily responsible for this effect of Ang II. Further supporting this conclusion was our finding...
that in cPLA\(_{\alpha}\)−/− BALB/c and C57BL/6 mice, reconstitution of cPLA\(_{\alpha}\) in the SFO by transduction with Ad-cPLA\(_{\alpha}\) DNA but not Ad-GFP DNA increased cPLA\(_{\alpha}\) expression and phospho-cPLA\(_{\alpha}\) immunoreactivity, and restored the effect of Ang II to increase BP. That cPLA\(_{\alpha}\) protein formed by transduction with Ad-cPLA\(_{\alpha}\) DNA, but not Ad-GFP DNA is capable of releasing AA has been confirmed in vascular smooth muscle cells. The decrease in the expression of cPLA\(_{\alpha}\) in the SFO transduced with Ad-Scr shRNA in cPLA\(_{\alpha}\)−/− mice, and the increase with Ad-cPLA\(_{\alpha}\) DNA in cPLA\(_{\alpha}\)−/− mice was selective because its expression in the PVN, heart and kidney were not altered in these mice. Ang II stimulates ROS production and ER stress in the SFO that leads to an increase in BP, most likely by increasing sympathetic activity. The increase in BP produced by Ang II 600 ng/kg/min, which is comparable to that obtained in the present study by 700 ng/kg/min of this peptide, is prevented by intracerebral ventricle administration of superoxide scavenger Ad-CuZn superoxide dismutase. Since (i) depletion of cPLA\(_{\alpha}\) by Ad-cPLA\(_{\alpha}\) shRNA in the SFO of cPLA\(_{\alpha}\)−/+ mice reduced, and (ii) expression of cPLA\(_{\alpha}\) by transduction with Ad-cPLA\(_{\alpha}\) DNA in cPLA\(_{\alpha}\)−/− BALB/c mice restored Ang II-induced ROS production and ER stress, this suggests that cPLA\(_{\alpha}\) expression and activity mediates the effect of Ang II on ROS production and ER stress. Whether alteration in cPLA\(_{\alpha}\) activity by Ang II in SFO also affects the ROS production and ER stress in PVN and rostral ventrolateral medulla remains to be determined. Ang II is known to produce cardiac and renal fibrosis, which is dependent on prohypertensive eicosanoids generated by cPLA\(_{\alpha}\). Our demonstration that Ang II-induced cardiac and renal fibrosis, as indicated by collagen accumulation, was minimized by depletion of cPLA\(_{\alpha}\) in the SFO by transduction with Ad-cPLA\(_{\alpha}\) shRNA in cPLA\(_{\alpha}\)−/+ BALB/c mice suggests that cPLA\(_{\alpha}\) activation in the SFO contributes to this action of Ang II. Supporting this view was our observation that reconstititution of cPLA\(_{\alpha}\) by Ad-cPLA\(_{\alpha}\) DNA in cPLA\(_{\alpha}\)−/− BALB/c mice caused Ang II to produce cardiac and renal fibrosis. Whether attenuation of Ang II-induced cardiac and renal fibrosis was caused by decreased expression of cPLA\(_{\alpha}\) by Ad-cPLA\(_{\alpha}\) shRNA in the SFO of cPLA\(_{\alpha}\)−/+ mice and restoration of fibrosis in these tissues by expression of cPLA\(_{\alpha}\) by Ad-cPLA\(_{\alpha}\) DNA in cPLA\(_{\alpha}\)−/− mice, which could be due to changes in BP and/or sympathetic activity, remains to be determined.

cPLA\(_{\alpha}\) activation by Ang II releases AA that is metabolized by COX, lipoxygenase, and cytochrome P450A into eicosanoids with prohypertensive and antihypertensive effects. Previously, we reported that prohypertensive eicosanoids generated by cPLA\(_{\alpha}\) activation contributed to Ang II-induced hypertension and associated cardiac and renal pathogenesis. COX-1 inhibitor SC560 minimized Ang II-salt-induced hypertension which is associated with the increased sympathetic activity. Decrease in COX-2 expression by IL-10 in PVN is related to reduced neuronal sympathetic excitation in heart failure in rats after myocardial infarction. On the other hand, proinflammatory cytokines stimulate COX-2 expression in perivascular macrophages, and when injected in the SFO increase BP, heart rate, and renal sympathetic activity. Therefore, Ang II via production of proinflammatory cytokines could increase COX activity and PGE2 synthesis. Reduction in COX-1 and COX-2 expression by their respective siRNA in PVN also reduces deoxycorticosterone-induced hypertension. COX-generated AA metabolite PGE2 injected into the cerebroventricular system or rostral ventrolateral medulla increases BP and sympathetic activity via EP3 receptors, respectively. Ang II-induced increase in BP is inhibited in both EP1 and EP3 receptor knockout mice or by EP1 and EP3 receptor antagonists. PGE2 generated by COX-2 in the SFO via the EP1 receptor is required for ROS generation and hypertension caused by Ang II. AA-metabolizing enzymes are constitutively active, and the rate-limiting step in the production of eicosanoids is the availability of AA. Therefore, cPLA\(_{\alpha}\) activation by Ang II in the SFO appears to be critical for AA release resulting in the production of PGE2, and generation of ROS and ER stress that increases BP and results in cardiac and renal fibrosis. The contribution in Ang II-induced hypertension of cPLA\(_{\alpha}\) in the PVN and rostral ventrolateral medulla where PGE2 via EP3 receptors increases BP remains to be investigated and is one of the limitations of the present study. Like in our study in cPLA\(_{\alpha}\)−/− mice, the COX1 or EP1 receptor gene disruption

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**Figure 4.** Ang II increases endoplasmic reticulum (ER) stress marker expression in the subfornical organ (SFO) of BALB/c cPLA\(_{\alpha}\)−/+ but not cPLA\(_{\alpha}\)−/− mice. RNA was isolated from SFO, and real-time PCR (RT-PCR) was performed for glucose-related protein 78 (GRP78), and C/EBP homologous protein (CHOP). (a) mRNA expression of GRP78 and CHOP in SFO of cPLA\(_{\alpha}\)−/+ mice. (b) mRNA expression of GRP78 and CHOP in SFO of cPLA\(_{\alpha}\)−/− mice. *P < 0.05, Ang II vs. Veh (vehicle) (n = 4). Data are expressed as mean ± SEM.
or the central administration of their pharmacological inhibitors attenuated the increase in BP produced by Ang II (600 ng/kg/min) that was comparable to that obtained in the present study. At present, we have no explanation how the central cPLA\(_2\)/COX1/EP1 receptor in SFO masks the direct vasconstrictor effect of Ang II. Further studies are required to determine if alteration in cPLA\(_2\)/COX/EP receptors in SFO and other brain areas also prevent the effect of bolus injections or short-term infusion of Ang II.

An important finding in our study was that Ang II also failed to increase BP in the partially cPLA\(_2\) gene-disrupted mice (cPLA\(_2\)+/−) expressing reduced cPLA\(_2\) mRNA in the heart, kidney, and SFO in C57BL/6 mice. These observations further support the critical role of cPLA\(_2\) in Ang II-induced hypertension. Further studies on different levels of cPLA\(_2\) expression or its copy number in the SFO and other tissues should allow the determination of its relationship to BP in various models of hypertension and associated pathogenesis. cPLA\(_2\) gene disruption also prevented hypertension produced by the inhibitor of nitric oxide synthesis, L-NG-nitroarginine methyl ester, that is dependent on Ang II.

Our preliminary data obtained in C57BL/6 mice showed that cPLA\(_2\) gene disruption abolished deoxy corticosterone-acetate-salt-induced hypertension and associated cardiac and renal fibrosis (C Y Song and K U Malik, unpublished results).

In conclusion, this study demonstrates that cPLA\(_2\) in the SFO is crucial in mediating the effect of systemic Ang II to cause ROS production and ER stress and hypertension, most likely by releasing AA and metabolizing it via COX producing PGE2. Our finding that the partial cPLA\(_2\) gene disruption (cPLA\(_2\)+/− mice) also prevented Ang II-induced hypertension supports the notion that cPLA\(_2\) activation is pivotal for the development of Ang II-induced hypertension. Therefore, development of selective orally active inhibitors of cPLA\(_2\) could be useful in the treatment of hypertension and its pathogenesis.

**SUPPLEMENTARY MATERIAL**

Supplementary materials are available at American Journal of Hypertension online.

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**DISCLOSURE**

The authors declared no conflict of interest.

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