Renal lysophospholipase A1 contributes to Enterococcus faecalis-induced hypertension by enhancing sodium reabsorption

Yuting Liu, Qing Zhu, Yufeng Tao, ..., Chun Zhang, Yang Chen, Lei Wang

ychen8@gzucm.edu.cn (Y.C.)
wanglei2017@gzucm.edu.cn (L.W.)

**Highlights**

- *E. faecalis* increased BP, serum angiotensin II, and sodium reabsorption in mice
- LYPLA1 contributed to *E. faecalis*-induced hypertension by elevating sodium reabsorption
- LYPLA1 elevated sodium reabsorption by accumulating GPC in the renal medulla
- LYPLA1 was enriched in the renal medulla and/or urine of other hypertensive animals

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Renal lysophospholipase A1 contributes to Enterococcus faecalis-induced hypertension by enhancing sodium reabsorption

Yuting Liu,1,5 Qing Zhu,2,5 Yufeng Tao,1 Yuting Zeng,1 Shasha Li,3 Liangyu Zeng,1 Chun Zhang,4 Yang Chen,1,6,* and Lei Wang1,4,7,*

SUMMARY

Our recent study has found that gut bacteria Enterococcus faecalis contributes to hypertension and upregulates lysophospholipase A1 (LYPLA1) in the renal medulla of rats. This work aimed to investigate the role of LYPLA1 in the development of E. faecalis-induced hypertension. Compared to control, E. faecalis treatment increased blood pressure (BP), serum angiotensin II, sodium reabsorption, and expression of αENaC and LYPLA1 in the renal medulla of mice, and these effects were attenuated by knockdown of LYPLA1. Moreover, the intrarenal lypla1 overexpression increased sodium reabsorption and BP. Further studies showed that LYPLA1 promoted the accumulation of renal glycerophosphocholine (GPC), which directly elevated the expression of αENaC and sodium reabsorption. In addition, enriched abundance of LYPLA1 in the renal medulla and urine was also observed in other hypertensive animals. Overall, our results demonstrate that LYPLA1 contributes to E. faecalis-induced hypertension by accumulating GPC and activating ENaC in the renal medulla.

INTRODUCTION

Epithelial sodium channel protein (ENaC) mainly locates at the collecting ducts and plays a critical role in the regulation of the final renal sodium excretion (Frindt et al., 2007; Staruschenko, 2012). Overactivation of ENaC disrupts systemic sodium homeostasis and further contributes to the development of hypertension (Mutchler et al., 2021). ENaC is composed of α, β, and γ subunits. All of them are essential to form functional channels for transepithelial Na+ transport, among which αENaC acts as a functional unit whereas βENaC and γENaC regulate the transport activity of the channel (Pavlov and Staruschenko, 2017). Increased expression and activity of βENaC and γENaC subunits are detected in the renal medulla and cortex of salt sensitive Dahl hypertensive rats and deoxycorticosterone acetate/salt (DOCA/salt) treated rats, which are regulated by aldosterone and the transport activity of the channel (Pavlov and Staruschenko, 2017). Increased expression and activity of βENaC and γENaC subunits are detected in the renal medulla and cortex of salt sensitive Dahl hypertensive rats and deoxycorticosterone acetate/salt (DOCA/salt) treated rats, which are regulated by aldosterone signaling (Kakizoe et al., 2009; Pavlov et al., 2013; Wu et al., 2021). In contrast, in angiotensin II (Ang II)-infused hypertensive animals, it is observed that sodium retention and blood volume expansion are accounted for (Feng et al., 2021; Peng et al., 2017; Wang et al., 2001). Abundant studies demonstrate that ENaC plays a vital role in the pathogenesis of hypertension but its regulatory mechanism is still incompletely understood.

Glycerophosphocholine (GPC) is an important organic osmotic adjustment substance, which abundantly exists in the medulla tissue of mammalian kidney, especially in the medullary collecting duct cells. GPC in cells balances osmotic pressure and maintains the hyperosmotic environment of renal medulla (Gallazini and Burg, 2009). The level of intracellular GPC is regulated by its enzymatic synthesis or degradation in response to high sodium salt or urea (Gallazini et al., 2006, 2008; Okazaki et al., 2019; Sonkar et al., 2019; Topanurak et al., 2013). Lysophospholipases play an important role in producing GPC in cells (Gallazini and Burg, 2009; Sonkar et al., 2019; Wang and Dennis, 1999). Actually, lysophospholipases can be divided into two sub-classes based on their molecular weights, including high molecular weights of >50 kDa and low molecular weights of <30 kDa (Wang and Dennis, 1999). Low molecular weight lysophospholipases can be sub-divided into lysophospholipases A1 (LYPLA1) and lysophospholipases A2 (LYPLA2). Both LYPLA1 and LYPLA2 are expressed in multiple organs and tissues including liver, heart, and kidney (Sugimoto et al., 1996; Wang and Dennis, 1999; Wang et al., 1999). A significant body of work has reported that high molecular weight lysophospholipases have regulatory effects on the GPC levels in cells and tissues.
Our recent work revealed that gut bacteria *E. faecalis* contributes to hypertension and renal injury in rats (Zhu et al., 2021). In that study, we found that the *E. faecalis* treatment increased blood pressure (BP) and serum Ang II levels but had no effects on serum endotoxin of rats. Further exploration showed that the administration of *E. faecalis* disturbed lipid metabolism in the serum and expression of lipid-metabolic enzymes in the kidney. Especially, the expression of LYPLA1 but not LYPLA2 was markedly elevated in the renal medulla of *E. faecalis*-induced hypertensive rats. Similar expression pattern of LYPLA1 was also reported in the renal medulla of high-salt fed Dahl salt-sensitive rats (Xue et al., 2018), suggesting it may be involved in sodium homeostasis. Hence, we hypothesized that renal LYPLA1 may contribute to the pathogenesis of *E. faecalis*-induced hypertension by regulating sodium homeostasis. To prove it, the *lypla1* knockdown or overexpression was conducted in vivo and in vitro to investigate its effects on the regulation of BP, sodium homeostasis and GPC accumulation.

**RESULTS**

**Accumulated LYPLA1 in the renal medulla of *E. faecalis*-induced hypertensive mice**

Compared to control, *E. faecalis* treatment resulted in elevated BP of mice from the 5th week. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) at the end of experiment were 134 ± 1 mmHg (vs 115 ± 1 mmHg, Figure 1A) and 90 ± 2 mmHg (vs 81 ± 2 mmHg, Figure 1A), respectively. Consistently, *E. faecalis*-treated mice exhibited higher serum Ang II levels than the control group (Figure 1B). As shown in Figures 1C and 1D, increased LYPLA1 were observed on the protein and mRNA levels in the kidney of *E. faecalis*-treated mice. Accumulated LYPLA1 was also found in the urine after *E. faecalis* treatment (Figure 1E). Consistent with our results in rats (Zhu et al., 2021), the immunohistochemical analysis showed that the LYPLA1 protein was induced by *E. faecalis* treatment only in the renal medulla but not the renal cortex (Figure 1F).

**Renal LYPLA1 knockdown abolished *E. faecalis*-induced hypertension and sodium reabsorption in mice**

To investigate the role of LYPLA1 in the kidney, intrarenal *lypla1* knockdown was conducted in mice (termed LYPLA1-rKD) and its successful knockdown was confirmed on the protein and mRNA levels (Figure 2A). Compared to vehicle *E. faecalis*-treated mice, BP was significantly attenuated in LYPLA1-rKD *E. faecalis*-treated mice (SBP: 111 ± 3 mmHg vs 132 ± 1 mmHg, DBP: 80 ± 2 mmHg vs 90 ± 3 mmHg, Figure 2B). Consistently, the serum levels of Ang II in LYPLA1-rKD *E. faecalis*-treated mice were lower than Vehl *E. faecalis*-treated mice (Figure 2C). Compared to vehicle group, *E. faecalis*-treated mice exhibited lower urinary Na⁺ levels, whereas these effects were reversed by the *lypla1* knockdown (Figure 2D). *E. faecalis*-decreased urine volume tended to be elevated by the *lypla1* knockdown but showed no significance (Figure 2E). Amiloride, ENaC specific blocker, significantly attenuated BP (SBP: 106 ± 1 mmHg versus 123 ± 1 mmHg; DBP: 89 ± 1 mmHg versus 79 ± 1 mmHg, Figure S1A) and increased sodium excretion in *E. faecalis*-treated mice (Figure S1B), suggesting a contributing role of ENaC in *E. faecalis*-induced hypertension. Because only increased αENaC expression was detected in the renal medulla of *E. faecalis*-treated mice (Figure S2), the protein levels of αENaC in the renal medulla were determined. As shown in Figure 2I, the increased protein levels of αENaC by the *E. faecalis* treatment was blunted by the *lypla1* knockdown.

Then, the levels of aldosterone in the serum and urine of mice were tested and the results showed that there were not any differences in all groups (Figures S3A and S3B), suggesting that these regulatory effects of LYPLA1 on sodium reabsorption and αENaC are not associated with aldosterone.

No significant differences were detected on BP, urinary Na⁺ levels or αENaC expression between vehicle and LYPLA1-rKD groups.

**Intrarenal overexpression of LYPLA1 contributed to hypertension and sodium reabsorption in mice**

Next, intrarenal overexpression of LYPLA1 was introduced into the renal medulla of mice (termed LYPLA1-rOE) and its successful overexpression was shown in Figure 3A. Compared to mice transfected with empty
vector (V-ctrl), LYPLA1-rOE mice presented higher SBP from the 5th week and the SBP at the end of 6 weeks was 122 ± 2 mmHg (vs 107 ± 2 mmHg, Figure 3B). Compared to V-ctrl group, the Na⁺ levels were found lower in the urine only after the 6-week intrarenal overexpression of LYPLA1 (Figure 3C); there were no significant difference of urine volume between groups (Figure 3D). Correspondently, only enhanced mRNA of αENaC were detected in the renal medulla of 6-week LYPLA1-rOE mice (Figures 3E–3G). The increased expression of αENaC only after 6 weeks was confirmed on the protein level in the renal medulla (Figure 3H). Consistent with lypla1 knockout, the overexpression of LYPLA1 had no regulatory effects on the levels of aldosterone in the serum and urine (Figures S3C and S3D).

To further confirm the effect of LYPLA1 on sodium absorption, 4% high salt diet (HS) was used to feed LYPLA1-rOE mice. As shown in Figure 3I, SBP of LYPLA1-rOE mice fed with HS presented a significant increase from the 2nd week and this increase maintained to the end of 4 weeks (LYPLA1-rOE + HS: 134 ± 3 mmHg; LYPLA1-rOE: 111 ± 2 mmHg; V-HS: 111 ± 1 mmHg), compared to LYPLA1-rOE mice or mice only fed with HS (Figure 3I).

Taken together, the above results demonstrate that renal LYPLA1 contributes to the elevation of BP and sodium reabsorption in mice.
LYPLA1 enhanced the expression and activation of ENaC in mouse inner medullary collecting duct cells

Whether LYPLA1 has a regulatory effect on the activity of ENaC was studied in the mouse inner medullary collecting duct cells mIMCD-K2. Compared to control, the protein levels of LYPLA1 and αENaC were markedly elevated in Ang II-treated cells (Figure 4A), but this elevation on αENaC expression were blocked by lypla1 knockdown (LYPLA1+/−/C0) (Figure 4B). Then the activity of ENaC was assessed by the Na+ transport across the apical membrane ([Na+]i) using SBFI (Figure 4C). Compared to control, Ang II-pretreatment increased the initial levels of [Na+]i whereas this increase was abolished by amiloride and lypla1 knockdown (Figure 4D). Extra 120 mM NaCl led to further increase of [Na+]i in Ang II-treated cells, still higher than those in other treatments (Figure 4E). These results suggested that the lypla1 knockdown blocked Ang II-activated ENaC. No significant difference was found between Vehl and lypla1 knockdown cells without Ang II-pretreatment.
To further confirm this result, *lypla1* was then overexpressed in mIMCD-K2 cells (LYPLA1OE), in which enhanced protein expression of αENaC was detected (Figure 5A). Correspondently, the *lypla1* overexpression increased [Na⁺]i before and after 120 mM NaCl treatment compared to empty vector control, which was diminished by amiloride (Figures 5B–5D).

Collectively, these findings suggest that LYPLA1 has a role in activating ENaC in renal collecting duct cells.

**LYPLA1-mediated GPC accumulation enhanced sodium reabsorption**

To verify whether GPC production is mediated by LYPLA1 in mIMCD-K2 cells, LC-MS/MS and ELISA analysis were used to determine GPC levels in *lypla1*-overexpressed cells. As shown in Figure 6A, GPC levels were significantly enhanced after the *lypla1* overexpression.
easy-manipulated, it was used to examine GPC content in the following experiments. Compared to V-ctrl group, renal GPC levels was significantly elevated after intrarenal lypla1 overexpression at the end of 6 weeks (Figure 6B). Although the lypla1 knockdown only did not decrease GPC levels, Ang II-induced GPC accumulation was blunted in lypla1-knockdown cells (Figure 6C). Similar phenomena were observed in vivo. Compared to Veh E. faecalis-treated group, the intrarenal lypla1 knockdown reduced GPC levels in the kidney in response to E. faecalis treatment (Figure 6D). The lypla1 knockdown only had no regulatory effect on renal GPC levels (Figure 6D). The above results suggest that LYPLA1 contributes to GPC accumulation in the kidney.

To further investigate whether GPC is involved in the regulation of sodium reabsorption, GPC was used to treat mIMCD-K2 cells. As shown in Figures 7A and 7B, 10^{–8} mol/L of GPC enhanced the protein levels of αENaC and this induction was started from 8-h treatment. Consistently, GPC significantly upregulated [Na\(^+\)]\(_i\) which was blocked by amiloride (Figures 7C–7E), suggesting a promoting effect of GPC on the activation of ENaC.
Moreover, the intrarenal infusion of GPC decreased urinary Na⁺ excretion (Figure 7F) and enhanced the protein expression of αENaC in mice (Figure 7G). These effects were reversed by amiloride (Figures 7F and 7G).

Taken together, these results demonstrated that LYPLA1-mediated GPC accumulation in the renal medulla contributes to the activation of ENaC and the aggravation of sodium reabsorption.

The abundance of LYPLA1 in other hypertensive animals

The abundance of LYPLA1 in the kidney and urine were also examined in other hypertensive animals. As shown in Figures 8A and 8B, LYPLA1 was induced in the renal medulla and urine of Ang II-infused rats. Accumulated LYPLA1 was also observed in the urine of high fructose/salt (HFS)-induced hypertensive mice and DOCA/salt-fed rats (Figures 8C and 8D). These results indicate that LYPLA1 may have roles in the pathogenesis of hypertension in various hypertensive animals.

DISCUSSION

As a lipid-metabolizing enzyme, the increased expression of LYPLA1 in the renal medulla has been reported in high salt-fed Dahl salt-sensitive rats (Xue et al., 2018) and E. faecalis-induced hypertensive rats (Zhu et al., 2021), but the role of LYPLA1 in the kidney is poorly defined. In this work, we found that the lypla1 knockdown in the kidney dramatically blocked E. faecalis-induced hypertension and sodium reabsorption (Figure 2). Comparatively, the intrarenal lypla1 overexpression enhanced sodium reabsorption and led to hypertension in mice (Figure 3). Mechanically, LYPLA1 contributed to GPC accumulation in renal medullary cells (Figures 4, 5, 6, and 7), which directly increased the expression and activity of ENaC. Our data in vivo and in vitro clearly demonstrate that LYPLA1 promotes hypertension by enhancing the GPC accumulation and ENaC activation in the renal medulla of E. faecalis-treated mice. However, whether there is a feedback effect of LYPLA1 on serum Ang II is still unclear. The potential mechanism that LYPLA1 functions on sodium reabsorption and E. faecalis-induced hypertension is shown in Figure 8E.

Recent clinical studies have revealed that LYPLA1 is an Ang II-related protein in the urine of patients with chronic kidney diseases (Konvalinka et al., 2016) and kidney transplantation (Mohammed-Ali et al., 2019). Higher LYPLA1 levels in the urine were also detected in E. faecalis-treated mice (Figure 1D), Ang II-infused...
rats (Figure 8B), HFS-induced hypertensive mice (Figure 8C) and DOCA/salt-fed rats (Figure 8D). It is unclear whether LYPLA1 contributes to increase BP in these different hypertensive animals. We notice that the serum level of Ang II is increased in Ang II, *E. faecalis* (the present work and (Zhu et al., 2021)), and HFS treatment (Chen et al., 2020a; Zhu et al., 2022) but inhibited in DOCA/salt (Basting and Lazartigues, 2017) and Dahl models (Pelisch et al., 2011). These imply that LYPLA1 may respond to risk stimulators other than Ang II, such as high salt or aldosterone. Other mechanism may work on the development of Ang II-independent hypertension, which needs further investigation to clarify.

Based on the previous studies, LYPLA1 is a cytoplasmic serine hydrolase with lysophospholipase, thioesterase and/or phospholipase activities. For examples, LYPLA1 from human brain and rat liver only acts as lysophospholipase to hydrolyze lysophospholipids (Sugimoto et al., 1996; Wang et al., 1999) which are normal constituents of cell membranes and play essential roles in many physiological and pathological processes (Tan et al., 2020); the one cloned from human kidney cancer cells HEK293 has much higher thioesterase activity than lysophospholipase activity, which is responsible for eliminating palmitoylated protein and lysophospholipids (Hirano et al., 2009); the one in the mouse endothelial cells presents thioesterase activity to improve vascular remodeling in chronic ischemia (Wei et al., 2020); a copy from rabbit renal cortex exhibits not only lysophospholipase activity but also calcium independent phospholipase activities (Portilla et al., 1998) which is responsible for the release of arachidonic acid (Leslie, 2015). Although these enzymes from different species share high sequence homology, their functions may be mainly determined by the activities they bear. In this work, our results suggested that renal LYPLA1 may have a lysophospholipase activity to upregulate the levels of GPC. However, it is still unknown whether this enzyme from the mouse renal medulla has other activities. More investigation is needed to understand the activities and roles of LYPLA1 in the regulation of ENaC and sodium reabsorption.

GPC has been recognized as an intermediate product of choline metabolism for decades of years (Sonkar et al., 2019). Cellular GPC can be regulated by choline or some intermediate which are used for the substrate to synthesize GPC (Nakanishi and Burg, 1989). On the other hand, GPC is affected by osmotic pressure (Gallazzini et al., 2008; Okazaki et al., 2019; Topanurak et al., 2013). Mechanically, this regulation is dependent on the activation of enzymes that produce or degrade GPC. A single phospholipase B (neuropathy target esterase, NTE) is the first identified to participate in cellular GPC synthesis (Gallazzini and Burg, 2009; Gallazzini et al., 2006). The expression of NTE is significantly enhanced by high NaCl (Gallazzini et al., 2006). Comparatively, the enzymatic degradation of GPC, conducted by glycerophosphodiesterase

**Figure 6. Effects of LYPLA1 on the levels of GPC in vitro and in vivo**

(A) GPC levels detected by LC-MS/MS analysis or ELISA in mIMCD-K2 cells with lypla1 overexpression (n = 5–6 per group).

(B) GPC levels in the kidney of mice after lypla1 overexpression (n = 5 per group).

(C) GPC levels in Ang II-treated cells with or without lypla1 knockdown (n = 6 per group).

(D) GPC levels in the kidney of *E. faecalis*-treated mice with or without lypla1 knockdown (n = 6 per group). Data are presented as mean ± SE. *p < 0.05, **p < 0.01; ns, no significance.
phosphodiesterase domain containing 5 (GDPD5), is inhibited by both high NaCl and urea (Gallazzini et al., 2008; Okazaki et al., 2019). In this work, we found that the overexpression of LYPLA1 enhanced the accumulation of GPC in vivo and in vitro (Figure 6), suggesting that LYPLA1 plays a role in the regulation of GPC production. However, the situation after lypla1 knockdown was not fully consistent. Although abolishing the increase by E. faecalis or Ang II treatment, only renal lypla1 knockdown did not result in a decrease of GPC levels in the kidney and mIMCD-K2 cells (Figures 6D and 6E); 4-week LYPLA1 overexpression had no increasing effects on GPC content in the renal medulla of mice. We suspect that it may be related to a complex balancing mechanism of GPC synthesis and degradation. Extending studies on the effects of LYPLA1 on enzymes that produce or degrade GPC would help understand the roles and underlying mechanism of LYPLA1 in kidney cells.
In the collecting duct, the final excretion of sodium is determined by ENaC (Frindt et al., 2007; Staruschenko, 2012). Although responsible for reabsorption of <5% of sodium-filtered load, ENaC plays a key role in regulation of fluid and electrolyte balance in the body. Recent studies have shown that the regulation of ENaC seems different between the cortical collecting ducts and the inner medulla (Feng et al., 2021; Peng et al., 2017; Wang et al., 2021). In response to Ang II infusion, only expression of αENaC but not β- or γ-subunit was elevated in the renal inner medulla. This increase of αENaC expression is mediated by activation of intrarenal renin-angiotensin system and contributes to sodium imbalance and hypertension, which is not observed in the renal cortex and independent on aldosterone. Similar phenomenon was detected in this work. We found that the E. faecalis treatment enhanced the expression of αENaC in the renal medulla of mice (Figure S2) and had no regulatory effects on aldosterone (Figure S3). Our data suggested that LYPLA1/GPC pathway promotes sodium reabsorption by increasing the expression and activation of αENaC in the renal medulla of E. faecalis-treated mice (Figures 2, 3, 4, 5, 6, and 7).

Figure 8. The LYPLA1 levels in the kidney or urine of Ang II, HFS, DOCA/salt-induced hypertensive animals and potential mechanism on hypertension
(A) Representative immunochemistry staining of LYPLA1 in the kidney (100x and partial magnification) and summarized intensities of their positive staining in Ang II-infused rats (n = 5–6 per group, bar = 100 µm).
(B) Urinary LYPLA1 contents in Ang II-infused rats (n = 6 per group).
(C) Urinary LYPLA1 contents in HFS-fed mice (n = 6 per group).
(D) Urinary LYPLA1 contents in DOCA/salt-fed rats (n = 8 per group).
(E) Potential mechanism of LYPLA1 exerted on hypertension. Data are presented as mean ± SE. *p < 0.05, **p < 0.01; ns, no significance.
It is known that ENaC also has critical roles in the regulation of potassium homeostasis and body fluid beyond classical transepithelial Na⁺ transport mechanisms (Mutchler et al., 2021; Ray et al., 2021; Rotin and Staub, 2021). As an upstream regulator, it is still secret whether LYPLA1 has similar effects on epithelial K⁺ and fluid transport. Previous studies showed that LYPLA1 can act as a thioesterase to regulate the calcium-activated potassium (BK) channels by depalmitoylation (McClafferty et al., 2020) or diacylation (Tian et al., 2012) in HEK293 cells. Because we did not pay attention to K⁺ levels or channels in this work, more exploration is needed to identify the regulatory roles of LYPLA1 on K⁺ homeostasis. In addition, there is no study to report the effects of LYPLA1 on fluid transport. In the present study, we did not observe any significant changes of urine volume in response to lypla1 knockdown or overexpression in mice (Figures 2E and 3D). Thus, it would be interesting to further investigate the functions of LYPLA1 in the kidney.

Limitations of the study
The present study investigated the role and mechanism of LYPLA1 in the development of E. faecalis-induced hypertension in mice. Our data demonstrate that LYPLA1 promotes hypertension by accumulating GPC and then overactivating ENaC in the renal medulla of E. faecalis-treated mice. However, there are still some limits in this work: (1) We used gene editing to investigate roles of LYPLA1 in the renal medulla. Actually, conditional knockout mice of renal collecting duct LYPLA1 could be a more precise tool to verify our findings. (2) Tail-cuff BP measurement is not very precise for hypertensive studies. (3) This work focused on the role of LYPLA1 in E. faecalis-induced hypertension but did not explore how E. faecalis increases BP or serum Ang II levels in mice. (4) We did not include a cleaved band for αENaC or other post-transcriptional modification for ENaC units in this study. Thus, it is of great interest to further explore and understand the roles of renal LYPLA1 in the pathogenesis of hypertension.

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SUPPLEMENTAL INFORMATION
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AUTHOR CONTRIBUTIONS
During the research work, L.W., Q.Z., and Y.C. designed the studies and drafted the manuscript; Y.L. did molecular and ELISA tests in tissues and cells. Q.Z., Y.L., Y.T., Y.Z., and L.Z. participated in animal
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STAR METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Antibodies          |        |            |
| Rabbit polyclonal anti-LYPLA1 | Affinity Biosciences | Cat# DF3737; RRID:AB_2836101 |
| Rabbit polyclonal anti-α-ENaC | Affinity Biosciences | Cat# DF9199; RRID:AB_2842395 |
| Rabbit polyclonal anti-β-ENaC | Affinity Biosciences | Cat# DF6601; RRID:AB_2838563 |
| Rabbit polyclonal anti-γ-ENaC | Affinity Biosciences | Cat# DF8540; RRID:AB_2841744 |
| Rabbit polyclonal anti-GAPDH | Affinity Biosciences | Cat# AF7021; RRID:AB_2839421 |
| Goat Anti-Rabbit IgG (H + L) HRP | Affinity Biosciences | Cat# S0001; RRID:AB_2839429 |

| Chemicals, peptides, and recombinant proteins |        |            |
| Glycerophosphocholine | Macklin | Cat# C824554 |
| Puromycin | Sigma-Aldrich | Cat# P8833 |
| Angiotensin II | Sigma-Aldrich | Cat# A9525 |
| Amiloride hydrochloride | MedChemExpress | Cat# HY-B0285A |
| in vivo-jetPEI | Polyplus Transfection | Cat# 101000040 |
| Vet bond tissue adhesive | 3M | Cat# 1469SB |
| Lipofectamine 3000 | Invitrogen | Cat# L3000008 |
| DMEM/F12 medium | Gibco | Cat# 11330032 |
| SBFI AM | Maokang biotechnology Co. Ltd | Cat# MX4509-100UG |
| Ang II ELISA Kit | Cloud-clone Corp. | Cat# CEA005Mu |
| GPC ELISA Kit | Jianglai biological | Cat# JL50597 |
| LYPLA1 ELISA Kit (rat) | Enzyme-linked Biotechnology Co. Ltd | Cat# ml284791-2 |
| LYPLA1 ELISA Kit (mouse) | Enzyme-linked Biotechnology Co. Ltd | Cat# ml517808-2 |
| Sodium ion concentration Assay Kit | Solarbio Science and Technology Co. Ltd | Cat# BC2805 |
| BCA protein assay reagent kit | GBCBIO Technologies | Cat# GS522 |
| HRP Conjugated Streptavidin | Boster | Cat# BA1088 |
| DAB Substrate Kit | Vector Laboratories | Cat# SK-4100 |

| Experimental models: Cell lines and animals |        |            |
| miMCD-K2 | The Institute of Hypertension, Sun Yat-sen University | N/A |
| male C57BL/6 SPF mice | Animal Center of Guangdong Province | N/A |

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Lei Wang (wanglei2017@gzucm.edu.cn).

Materials availability
Plasmids generated in this study will be made available upon request to the lead contact.

Data and code availability
- All dataset generated or analyzed during this study are included in the published article. Detailed datasets supporting the current study are available from the lead contact upon request.
- This paper does not report original code.
- Any additional information is available from the lead contact upon reasonable request.
EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals
Male C57BL/6 SPF mice (20–25 g, 6–8 weeks old) were purchased from the Animal Center of Guangdong Province (Guangzhou, China). Mice were housed in a temperature-controlled room with 12:12 h light–dark cycle. All animals were permitted ad libitum consumption of water and food (0.3% NaCl). The animal protocols in this study were approved by the Institutional Animal Care and Use Committee of Guangzhou University of Chinese Medicine.

mIMCD-K2 cell culture and treatment
The mouse inner medullary collecting duct cells mIMCD-K2 was received from the Institute of Hypertension, Sun Yat-sen University (Su et al., 2017). The mIMCD-K2 cells were routinely propagated in DMEM/F12 medium (Gibco, C11330500BT), supplemented with 10% fetal bovine serum and 1% Pen-Strep Solution. After incubation with serum-free DMEM/F12 medium for 1 h, the mIMCD-K2 cells were treated with Ang II (10^{-7} M, Sigma, A9525) or GPC (10^{-9}-10^{-5} M, Macklin, C824554). At the end of the experiments, cells were harvested for the following tests of Western blotting, ELISA or qRT-PCR.

METHOD DETAILS

Gene editing of lypla1
For lypla1 knockdown, single-guide RNA sequence (gRNA) of LYPLA1 was received from the CRISPR Design website (http://crispor.tefor.net/). Then two nucleotide sequences, CACC GTCCGATGCC CGCCGTTGTGC (sense) and AAAC GCACAACGGCGGGCATCGGA (antisense), were synthesized (TsingKe), annealed and inserted into the BbsI site of the commercial plasmid pX459, generating a vector pWMLYPLA1g. The gRNA sequence insertion was verified by DNA sequencing (TsingKe).

The lypla1 overexpression plasmid was constructed based on a commercial vector pEGFP-N1 (Invitrogen, V790-20) which carries a constitutive promoter. Briefly, the mouse lypla1 fragment was amplified with the primers CGGAATTCCGCTTCCGACGCACTGTC (sense) and CGCGGATCC GTCAATCAATTGGAGGT AGGAGC (antisense), then cloned into the EcoRI-BamHI site of pEGFP-N1, yielding the lypla1 overexpression plasmid pWMLYPLA1e. The constructed plasmid was confirmed by DNA sequencing (TsingKe).

Animal protocols

Protocol 1
Uninephrectomized mice were administered orally with E. faecalis (10^7 CFU/day) for 8 weeks. The ones were received with PBS as control. BP was recorded by tail-cuff method using a noninvasive BP instrument (Softron Beijing Biotechnology Co. Ltd, Beijing, China, BP-2010A). At least six stable results were used for each parameter and the average value was calculated. Amiloride (1.5 mg/kg/day, MedChemExpress, HYB0285A) was intraperitoneally injected to E. faecalis-treated mice. All the measurements were performed between 9:00 AM-12:00PM. At the end of animal experiments, kidneys were removed and cut for the following molecular tests and morphological analysis.

Protocol 2
The intrarenal knockdown of lypla1 in uninephrectomized mice was performed with pWMLYPLA1g as well as corresponding empty vector in combined with the transfection reagent in vivo-jetPEI (Polyplus Transfection, PT-101-01N), a polyethylenimine derivative, as we previously described (Chen et al., 2020b). Mice were randomly divided into four groups: (1) one group with lypla1 knockdown and E. faecalis administration (LYPLA1-rKD EF); (2) one group with lypla1 knockdown and PBS (LYPLA1-rKD); (3) one group with empty vector pX459 and E. faecalis administration (Vehl-EF); (4) one group with empty vector and PBS (Vehl). BP was recorded by tail-cuff method using a noninvasive BP instrument (Softron). All the measurements were performed between 9:00 AM-12:00PM. At the end of 8 weeks, 24-h urine samples of mice were harvested with metabolic cages. Serum samples and renal tissues were collected for the following molecular tests.

Protocol 3
The lypla1 overexpression plasmid was transfected into the kidney of uninephrectomized mice as we previously described (Chen et al., 2020b). Then mice were fed with normal diet and divided into two groups: (1)
one group with lypla1 overexpression (LYPLA1-rOE); (2) the other with empty plasmid pEGFP-N1 (V-ctrl). BP were measured by tail-cuff method for the next six weeks. All the measurements were performed between 9:00 AM-12:00PM. At the end of experiment, 24-h urine samples, serum samples and renal tissues were collected for the following molecular tests.

Protocol 4
To investigate whether high salt diet accelerate the development of hypertension in LYPLA1-rOE mice, mice were randomly divided into four groups: (1) one group with renal lypla1 overexpression and 4% sodium chloride (HS) diet (LYPLA1-rOE + HS); (2) one group with renal lypla1 overexpression and normal diet with 0.3% NaCl (LYPLA1-rOE); (3) one group with empty vector and HS diet (Vehl + HS); (4) one group with empty vector and normal diet with 0.3% NaCl (V-Ctrl). SBP was measured by tail-cuff method for the next four weeks. All the measurements were performed between 9:00 AM-12:00PM. At the end of experiment, 24-h urine samples, serum samples and renal tissues were collected for the following molecular tests.

Protocol 5
Renal GPC infusion was done in uninephrectomized mice. The left kidney of mice was exposed from the flank region and an interstitial infusion catheter was placed into the renal medulla, ~2–3 mm underneath the kidney surface, and secured using 3 mol/L Vet bond tissue adhesive (3M, 1469SB) and a small piece of fat tissue. 100 mg/kg of GPC or saline (200 ul) was infused into the renal medulla through the infusion catheter (10 μL/min). After infusion and suture, each mouse was intraperitoneally injected with 1 mL saline and put into metabolic cages to collect the 12-h urine samples. Amiloride (1.5 mg/kg, MedChemExpress, HY-B0285A) was given twice (one day and 1 h before the infusion) as needed.

Protocol 6
The kidney and urine samples of Ang II-infused, DOCA/salt-fed rats or high-fructose plus salt-fed (HFS) mice were received from our previous studies (Chen et al., 2020b; Zhang et al., 2019; Zhu et al., 2022). The urine samples were collected for the following examination.

Enzyme-linked immunosorbent assay (ELISA)
The levels of serum Ang II were quantified by an enzyme immunoassay test according to the manufacturer’s instruction (Cloud-clone Corp., Houston, TX, USA, CEA005Mu).

The levels of Na⁺ in the serum or urine were measured by a commercial kit (Beijing Solarbio Science and Technology Co. Ltd, Beijing, China, BC2805). According to the manufacturer’s instruction, each sample was diluted with absolute ethanol, mixed well, and centrifuged at 10,000rpm, 4°C for 10 min. Then 20 μL of supernatant was mixed with potassium pyroantimonate reagent to generate precipitation in a weakly alkaline solution. The optical density (OD) was measured at 520 nm and the Na⁺ content was calculated.

The levels of LYPLA1 in the urine were determined by an enzyme immunoassay test (Enzyme-linked Biotechnology Co. Ltd, Shanghai, China, ml517808 for mouse, ml284791 for rat). As described in the manufacturer’s instruction, 50 μL of urine was added to the wells pre-coated with the LYPLA1 specific antibody. The OD of each well was measured at 450 nm.

The levels of GPC in the cells, renal tissues were tested by a commercial enzyme immunoassay test (Jianglai biological, shanghai, China, JL50597). 10 μL of each sample was added to the wells pre-coated with GPC specific antibody. Finally, the OD of each well was measured at 450 nm.

Quantitative reverse transcriptase polymerase chain reaction
For quantitative reverse transcription polymerase chain reaction (qRT-PCR), total RNA was extracted from tissues or cells. Reverse transcription and SYBG qPCR were performed as the manufacturer’s instruction (TsingKe Biological Technology, Beijing, China, TSE202). qPCR was performed using a BioRad CFX96 qPCR System. The primers for mouse LYPLA1 are TTTCTCAGGGGCATCAAC and ATGAGCTTGCTCATGCTC; primers for mouse αENaC are GTTTCACTTTTACCTGTGTTT and CGGATGATGAGATGTGTTCT; primers for mouse βENaC are CAGTGGGGAGCTTCCATCC and TCCTGTTGTTGCTGTGTTCT; primers for mouse γENaC are CTGTTCTTGTGGGATG and GACACCAGGAAGGTTT; primers
for mouse GAPDH are ATGGTGAAGGTCGGTGTGAA and GGTCGTTGATGGCAACAATCTC. The fold-changes to GAPDH were determined using the comparative threshold cycle (Ct) method.

**Western blotting**

Cell or tissue samples were homogenized in RIPA lysis buffer (GBCBIO Technologies Inc., Guangzhou, China, G3424). After centrifugation, the total protein concentration in the supernatant was determined by a BCA protein assay reagent kit (GBC, G3422). 30 μg of protein for each sample was denatured in a metal heater at 95°C for 5 min, then separated by SDS-PAGE and transferred onto polyvinylidene difluoride (PVDF) membrane. The membrane was blocked within 5% nonfat dry milk in Tris-buffered saline for 2h, followed by incubated with rabbit anti-LYPLA1 antibody (1:1000 dilution, Affinity Biosciences LTD, Changzhou, China, DF3737) or anti-αENaC/βENaC/γENaC antibody (1:1000 dilution, Affinity, DF9199/DF6601/DF8540) at room temperature for 1h and then overnight at 4°C. For GAPDH, the membranes were stripped and reprobed with rabbit anti-GAPDH antibody (1:4000 dilution, Affinity, AF7021). After washing with Tris-buffered saline, membranes were incubated with goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (1:3000 dilution, Affinity, 50001) and visualized using enhanced chemiluminescence. The intensities of blotted bands were quantified with the software ImageJ (free download from http://rsbweb.nih.gov/ij/).

**Immunohistochemical analysis**

For immunostaining, 4μm paraffin kidney sections were incubated at 65°C for 60 min until wax melts. After deparaffinization and rehydration, the tissues were placed in 10 mM sodium citrate buffer (pH = 6.0) and heated in a microwave oven for antigen unmasking. Following incubation in 0.3% H2O2 diluted by 100% MeOH for 10 min, the sections were incubated at room temperature for 30 min in PBS containing 1% BSA (Sigma, 9048-46-8) to block nonspecific binding. Then the sections were incubated overnight at 4°C in a humidified chamber with an antibody against rabbit anti-LYPLA1 (Affinity, DF3737) diluted 1:500 in PBS containing 1% BSA. After three times wash, the slides were incubated for 30 min at room temperature with goat anti-rabbit IgG antibody (Vector Laboratories, BA–9200) and then HRP conjugated streptavidin (Boster, BA1088) in PBS. Then the sections were stained with diaminobenzidine (Vector Laboratories, SK4100) and counterstained with hematoxylin (LEAGene, DH0006). After dehydration, the tissues were mounted by permount histological neutral balsam. The extent of reactivity for brown staining was observed under a light microscope. The positive staining area was quantified using image analysis software (Image-ProPlus).

**lypla1 editing in mIMCD-K2 cells**

For lypla1 knockdown, pWMLYPLA1g was transfected into mIMCD-K2 cells by Lipofectamine 3000 transfection reagents (Invitrogen, L3000008). After 12 h, the transfected cells were incubated with 3 μg/mL puromycin (Sigma, P8833) in the media to screen out the cells containing pWMLYPLA1g. The clones with lypla1 knockdown (LYPLA1+/−) were verified by Western blotting.

For lypla1 overexpression, pWMLYPLA1e or pEGFP-N1 was transfected into mIMCD-K2 cells by Lipofectamine 3000 transfection reagents (Invitrogen, L3000008). The LYPLA1 overexpression was verified by Western blotting.

**Measurement of intracellular sodium**

We used measurements of [Na⁺], as a reflection of Na⁺ transport across the apical membrane using the MetaFluor imaging system and sodium-binding benzofuran isophthalate (SBFI, Maokang biotechnology Co. Ltd, shanghai, China, MX4509) according to the methods described before (Dvorzhak et al., 2016; Peti-Peterdi et al., 2002). Briefly, mIMCD-K2 cells were plated on a confocal dish at a density of 1 x 10⁶ cells/dish. After pre-treated by GPC (10⁻⁷ M), Ang II (10⁻⁷ M), amiloride (10⁻⁶ M) or transfected with plasmids, mIMCD-K2 cells were incubated with pre-configured culture medium (without serum) containing 5 μM SBFI probe, 0.02% Pluronic F-127, and 2.5mM probenecid for 1 h. Subsequently, the cells were incubated in serum-free medium for another 30 min and then washed 3 times with HEPES buffer [4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid (HEPES) 3.56 g/L, NaH2PO4·12H2O 0.52 g/L, pH7.4]. SBFI fluorescence was measured by at the ratio of an emission wavelength of 510 nm in response to excitation wavelengths of 340 and 380 nm (Rg/380). As the fluorescent signals were stable, the initial [Na⁺] of cells (R0) was recorded for each treatment. Then, extra 120mM NaCl was added to induce the elevation of
[Na\(^+\)], until the fluorescence reached extreme peak (peak fluorescence ratio, \(R_p\)). The [Na\(^+\)] response to high salt is defined as 
\[
\Delta R/R_0 = 100\% \times (R_p - R_0)/R_0.
\]

**GPC detection by LC-MS/MS analysis**

60 \(\mu\)L of cell extracts was mixed with 180 \(\mu\)L of methanol solution, vortexted for 30s and placed at −20°C for 30 min to precipitate proteins. After centrifugation at 12,000 \(g\) at 4°C for 15 min and filtered by 0.22-\(\mu\)m micromembrane, the supernatant was carefully transferred to sample vials for LC-MS/MS analysis. GPC was prepared in methanol at the concentration of 0.001–100 \(\mu\)g/mL to make a standard curve. Chromatography was performed on a Waters Acquity UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 \(\mu\)m) at 30°C. The mobile phase consisted of acetonitrile (Merck, Germany) as solvent A and 0.1% formic acid (Merck, Germany) in water as solvent B. 10 \(\mu\)L of each sample was injected and then the elution was performed in the solvent 0.1% formic acid/water (A, 20%) and methanol (B, 80%) for 5 min at a rate of 0.3 mL/min, respectively.

MS was performed on AB SCIEX Triple TOF 5600 system equipped with the Duo Spray source (AB Sciex, UK). The ESI probe was used for sample analysis and the APCI probe was used to perform automatic mass calibration through the calibrant delivery system (CDS). Information dependent acquisition (IDA) was used to automatically acquire MS/MS data when an MS signal exceeded a threshold of 3000 cps. MS spectra were acquired in positive ionization mode. The flow rate of ion source gas 1, ion source gas 2 and the curtain gas were set as 50 mL/min, 50 mL/min and 35 mL/min, respectively. The ion spray voltage was set to 5500V at ion source temperature of 500°C. A TOF-MS survey scan (50–1500 \(m/z\)) followed by 3 MS/MS scans (50–1500 \(m/z\)) with accumulation time of 0.25 and 0.1 s respectively. The declustering potential voltage was 80 V. For TOF MS, the collision energy was set to 10 V and set to 40 V with a spread of ±15 V. The ion release delay was 67 and the ion cluster width was 25. Dynamic background substrate (DBS) mode was used in this detection.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Data are presented as mean ± standard error (SE). Statistical analysis and graphing were performed by GraphPad Prism 8. The normal distribution of data was detected by Shapiro Wilk test. Two groups with normal distribution were analyzed by Student t tests. Comparison between three groups and above was analyzed by one-way ANOVA followed by Turkey or Dunnett post hoc multiple tests, or the nonparametric Kruskal Wallis tests. A P-value below 0.05 compared with control was considered statistically significant.