RESEARCH ARTICLE

Group V Secretory Phospholipase A2 Is Involved in Tubular Integrity and Sodium Handling in the Kidney

João Luiz Silva-Filho1,‡, Diogo Barros Peruchetti1,‡, Felipe Moraes-Santos1, Sharon Schilling Landgraf1,2, Leandro Souza Silva1, Gabriela Modenesi Sirtoli1, Daniel Zamith-Miranda1, Christina Maeda Takiya3, Ana Acaciu Sá Pinheiro1,4, Bruno Lourenço Diaz1, Celso Caruso-Neves1,5*

1 Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil, 2 Instituto Federal de Educação, Ciência e Tecnologia, Rio de Janeiro, RJ, Brazil, 3 Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil, 4 Instituto Nacional para Pesquisa Translacional em Saúde e Ambiente na Região Amazônica, Conselho Nacional de Desenvolvimento Científico e Tecnológico/MCT, Rio de Janeiro, RJ, Brazil, 5 Instituto Nacional de Ciência e Tecnologia em Biologia e Bioimagem, Rio de Janeiro, RJ, Brazil

☯ These authors contributed equally to this work.
‡ These authors share the first authorship on this work.
*
caruso@biof.ufrj.br

Abstract

Group V (GV) phospholipase A2 (PLA2) is a member of the family of secreted PLA2 (sPLA2) enzymes. This enzyme has been identified in several organs, including the kidney. However, the physiologic role of GV sPLA2 in the maintenance of renal function remains unclear. We used mice lacking the gene encoding GV sPLA2 (Pla2g5−/−) and wild-type breeding pairs in the experiments. Mice were individually housed in metabolic cages and 48-h urine was collected for biochemical assays. Kidney samples were evaluated for glomerular morphology, renal fibrosis, and expression/activity of the (Na+ +K+)-ATPase α1 subunit. We observed that plasma creatinine levels were increased in Pla2g5−/−mice following by a decrease in creatinine clearance. The levels of urinary protein were higher in Pla2g5−/−mice than in the control group. Markers of tubular integrity and function such as γ-glutamyl transpeptidase, lactate dehydrogenase, and sodium excretion fraction (FENa+) were also increased in Pla2g5−/−mice. The increased FENa+ observed in Pla2g5−/−mice was correlated to alterations in cortical (Na+ + K+) ATPase activity/ expression. In addition, the kidney from Pla2g5−/− mice showed accumulation of matrix in corticomedullary glomeruli and tubulointerstitial fibrosis. These data suggest GV sPLA2 is involved in the maintenance of tubular cell function and integrity, promoting sodium retention through increased cortical (Na+ + K+) -ATPase expression and activity.
Introduction

Phospholipase A2 (PLA2) is a superfamily of enzymes that provides free fatty acids and lysophospholipids from the hydrolysis of the ester bond at the sn-2 position of glycerophospholipids [1]. Currently, PLA2 enzymes have been classified into 6 types with 16 distinct groups according to their structure, function, and cellular location [1].

Secretory phospholipase A2 (sPLA2) was the first type of PLA2 discovered [2]. In mammals, this family contains 10 catalytically active isoforms, including the group V sPLA2 (GV sPLA2) [2]. GV sPLA2 plays an important role in diverse biological and pathological cellular processes due to its capacity to metabolize phospholipids and produce a free fatty acid and a lysophospholipid. Although it has been reported that GV sPLA2 promotes the release of arachidonic acid and subsequent generation of eicosanoids, such as prostaglandins and leukotrienes [1,2], this may not be relevant to its physiologic functions [3,4]. However, GV sPLA2 may still participate in the synthesis of eicosanoid through activation of GIVA PLA2 [5–7] or induction of cyclooxygenase (COX)-2 activity [8,9].

Several animal studies have revealed that GV sPLA2 contributes to eosinophilic pulmonary inflammation [10–12], abdominal aortic aneurysms [13], ischemic injury [14], and autoimmune diseases [15]. In addition, GV sPLA2 has also shown potent antibacterial and antiviral properties [16]. In this regard, several studies have elucidated the role of GV sPLA2 in different systems, particularly in pathological processes, but the function of this enzyme in the kidney, regulation of renal hemodynamics or involvement in kidney disease, remains unclear.

Expression of GV sPLA2 has been demonstrated in the kidney of rats [17] and mice [18]. It has also been shown that GV sPLA2 is constitutively expressed in the tubular epithelium of normal human kidneys and its expression is markedly upregulated in the tubules and glomeruli during kidney damage [14]. Studies on human embryonic kidney 293 cells (HEK293) and in primary cultures of mouse mesangial cells have also shown that GV sPLA2 amplifies the release and conversion of arachidonic acid into prostaglandins by increasing GIVA PLA2 and COX-2 activity [19,20]. However, the in vivo significance of the activity of GV sPLA2 on renal function has not been described.

In the present work, we used mice with a homozygous disruption in the gene encoding GV sPLA2 (Pla2g5−/−) to clarify the role of this PLA2 group on renal function. Our data revealed that GV sPLA2 plays a physiologic role in the maintenance of renal function and sodium handling, with a major influence on the tubular compartment rather than in the glomerulus.

Materials and Methods

Animals

Mice with targeted disruption of the gene encoding GV sPLA2 (Pla2g5−/−) were generated by Satake et al. [21]. We used 12-week-old male Pla2g5-null and wild-type (WT) mice in a C57BL/6 genetic background in all experiments. Mice were caged with free access to food and fresh water in a temperature-controlled room (22–24°C) with a 12-h light/dark cycle until used. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Ethics Committee of Federal University of Rio de Janeiro (permit number IBCCF004). For the euthanasia procedure, animals were anesthetized with ketamine (80 mg/kg body weight) and xylazine (5 mg/kg body weight) before blood collection via cardiac puncture.

Reverse transcription-polymerase chain reaction

Kidneys of WT and Pla2g5−/− mice were dissected and total RNA from the renal cortex and medulla was extracted using TRIZOL reagent (Invitrogen, Karlsruhe, Germany).
Contaminating genomic DNA was removed by DNase I (Fermentas, St. Leon-Rot, Germany) before reverse transcription (RT) of 1 μg of total RNA using a Superscript III kit (Invitrogen, Karlsruhe, Germany). To determine the expression of GV sPLA₂ in mice kidneys, cDNA was submitted to conventional polymerase chain reaction (PCR) using the following primers: forward AAC AGG CGC TGA GAC CAG, and reverse GAC ATT AGC AGA GGA AGT TGG G and settings: denaturation—95°C, annealing—53°C and extension – 72°C in a 35 cycles PCR reaction. The amplicons generated were resolved on agarose gel electrophoresis and analyzed under UV light. A band of the expected size (~455 bp) for GV sPLA₂ mRNA was observed in the cortex and medulla of kidneys obtained from WT mice [22]. On the other hand, RT-PCR analysis of Pla2g5−/− mice confirmed the lack of GV sPLA₂ mRNA in these animals (data not shown).

Measurement of renal function

Mice were kept individually in metabolic cages to analyze renal function. The cages were maintained in a temperature-controlled room (22–24°C) with a 12-h light/dark cycle, with free access to tap water and standard rodent diet. After 2 days of acclimatization, 48-h urine was collected to determine urine volume, total protein, creatinine, sodium, γ-glutamyl transpeptidase (γGT), and lactate dehydrogenase (LDH) concentrations. Before analysis, urine samples were centrifuged at 3000×g for 10 min to clear sediments. Blood samples were collected and centrifuged at 1200×g for 10 min at 4°C to obtain plasma to measure sodium and creatinine concentrations.

The levels of urinary protein were determined by the pyragallol red method (Gold Analisa kit #498M, Belo Horizonte, MG, Brazil) and creatinine by the alkaline picrate method (Gold Analisa kit #335, Belo Horizonte, MG, Brazil). Kits for γGT (Bioclin kit #K080, Belo Horizonte, MG, Brazil) and LDH (Gold Analisa kit #457, Belo Horizonte, MG, Brazil) were used for quantitative determination of the enzyme activity. Sodium levels were analyzed by the photometric colorimetric test (Human Diagnostics Worldwide kit #573351, Wiesbaden, Germany). Plasma and urine osmolality were measured on an Advanced Micro Sample Osmometer 3320 (Advanced Instruments, Norwood, MA).

Histologic and histomorphometric studies

Kidneys were fixed in a 4% buffered formalin solution and embedded in paraffin. Histologic sections (3-μm thick) of kidney were obtained and stained with periodic acid-Schiff reagent (PAS; Sigma-Aldrich, St Louis, MA) for analysis of the mesangial surface of subcapsular and corticomedullary glomeruli. In addition, 7-μm-thick sections were cut to assess the deposition of collagen fibers with Picrosirius Red staining (Sigma-Aldrich, St. Louis, MA). Only interstitial collagen was counted, and vessels and glomeruli were excluded. Data were expressed as a percentage of the interstitial area with positive staining. Quantification analysis of PAS and Picrosirius Red-stained sections were performed using Image-Pro Plus analysis software on 25 photomicrographs in a light microscope equipped with a camera (Eclipse E800, Nikon).

Preparation of the homogenate fraction

The homogenate fraction of the renal cortex and medulla was obtained as described previously [23]. Briefly, kidneys were removed and homogenized in a cold solution containing 250 mmol/l sucrose, 10 mmol/l HEPES–Tris (pH 7.6), 2 mmol/l EDTA, and 1 mmol/l phenylmethylsulfonyl fluoride. Homogenates were centrifuged at 7000×g at 4°C for 10 min and the final supernatant was stored at ~80°C. Protein concentrations were determined by the Folin phenol method [24] using bovine serum albumin as standard.
Immunoblotting

Proteins were resolved on sodium dodecyl sulfate-polyacrylamide gels and transferred to nitrocellulose membrane (Millipore Corporation, Bellerica, MA), according to the manufacturer’s instructions. The (Na\(^+\) + K\(^+\))-ATPase \(\alpha_1\) subunit was immunodetected in the homogenate fraction of the renal cortex and medulla with specific primary antibody (1:10 000; #05–369, Millipore Corporation, Bellerica, MA). After antibody labeling, detection was performed with ECL-plus (Amersham Biosciences, Piscataway, NJ).

Measurement of (Na\(^+\) + K\(^+\))-ATPase activity

ATPase activity was evaluated by spectrophotometric measurement of inorganic phosphate released from ATP with the use of ammonium molybdate as described by Maritno et al. [25]. The composition of the standard assay medium was 15 mM MgCl\(_2\), 5 mM ATP-Na\(^+\), Tris (pH 7.0), 150 mM NaCl, and 15 mM KCl. The reaction was started by the addition of 3 mg/ml protein from the renal cortex or medulla homogenate (final concentration, 0.3 mg/ml). After 10 min of incubation at 37°C, the reaction was stopped by the addition of trichloroacetic acid. Phosphate solutions (0–40 \(\mu\)M) were used as standards. The phosphate content was determined by measurement of absorbance at 660 nm. The (Na\(^+\) + K\(^+\))-ATPase activity was calculated as the difference in ATPase activity between renal cortex or medulla homogenate exposed to ouabain and those not exposed.

Statistical analysis

Each experiment was carried out using 4 animals per group. Data are reported as the mean ± standard error of at least 2 representative experiments. Statistical analysis was performed using Prism software (GraphPad Software, version 5), and, unless otherwise stated, means were compared by the two-tailed Student t test. The significance level was set at \(\alpha = 0.05\).

Results

GV sPLA\(_2\) is important to renal function homeostasis

To elucidate the physiologic role of GV sPLA\(_2\) on renal function, we measured related parameters in WT and \(\text{Pla2g5}^{-/-}\) mice (Table 1). The results show that urinary flow and creatinine clearance (CCr, a marker of glomerular flow rate) were decreased in \(\text{Pla2g5}^{-/-}\) mice compared with the WT group (Table 1). The decrease in CCr was followed by an increase in plasma creatinine in \(\text{Pla2g5}^{-/-}\) mice. Urinary osmolality (Uosm) was increased in \(\text{Pla2g5}^{-/-}\) mice without changes in plasma osmolality (Posm). Body weight was not changed in both WT and \(\text{Pla2g5}^{-/-}\) mice. The ratio of urinary protein to creatinine (UPCr), a marker of renal injury [26], was slightly higher in \(\text{Pla2g5}^{-/-}\) mice compared with WT animals. These results indicate that GV sPLA\(_2\) is important for the maintenance of renal function.

Mild glomerular morphologic changes in \(\text{Pla2g5}^{-/-}\) mice

Several studies have shown that a decline in the glomerular filtration rate can be correlated with glomerular morphologic changes [27]. Thus, we wondered whether the decreased CCr in \(\text{Pla2g5}^{-/-}\) mice is correlated to morphologic changes in the glomerulus. We analyzed the glomerular structure of WT and \(\text{Pla2g5}^{-/-}\) mice. The subcapsular and corticomedullary glomeruli of WT and \(\text{Pla2g5}^{-/-}\) groups were assessed by light microscopy (Fig 1). The mesangial surface was revealed by accumulation of PAS-positive material in mesangial area.
As shown in Fig 1, although the number of cells per glomerulus was not different, the mesangial surface of the corticomedullary glomeruli was increased in Pla2g5−/− mice compared with controls (Fig 1A–1C). Conversely, the mesangial surface of the subcapsular glomeruli and cellularity were not significantly different between the WT and Pla2g5−/− groups (Fig 1D–1F). Thus, these results indicate that changes in the CCr, observed in Pla2g5−/− mice, are not correlated to major glomerular morphologic alterations in these mice and may be only caused by changes in glomerular function.

GV sPLA2 is critical for the maintenance of tubular integrity

It is well known that glomerular injury and tubular impairment are involved in early events that lead to proteinuria [26]. Because no major changes in glomerulus structure seem to occur in Pla2g5−/− mice, we investigated if the higher UPCr observed in these knock-out mice could be associated with changes in renal tubular integrity and function.

LDH activity and γGT activity, markers of altered tubular integrity, were determined in urine (Fig 2A and 2B), and fibrosis was visualized by Picrosirius Red staining for collagen fibers (Fig 2C–2F). Fig 2A and 2B shows that urinary LDH and γGT activities were significantly increased in Pla2g5−/− mice in relation to control mice. A similar profile was observed in cortical interstitial fibrosis. Collagen deposition was enhanced in Pla2g5−/− mice compared with the WT group (Fig 2C and 2D). On the other hand, tubular interstitial space was not changed in the different mice groups (Fig 2E and 2F). These results suggest that GV sPLA2 is critical to conserve tubular integrity and the higher proteinuria observed in Pla2g5−/− mice may be associated with deficiency of this function.

GV sPLA2 promotes sodium retention

Previous studies have shown a positive correlation between tubular cell damage and the sodium excretion fraction (FE\textsubscript{Na\textsuperscript{+}}) [28], indicating impairment of tubular function. Thus, based on the aforementioned results suggesting a critical role of GV sPLA2 in the preservation of tubular integrity, we wondered whether this enzyme also affects tubular function. Because sodium handling is a hallmark of the tubular function, we verified some functional parameters related to renal sodium excretion in Pla2g5−/− and WT mice (Fig 3). Fig 3A and 3B shows that urinary sodium excretion (U\textsubscript{Na\textsuperscript{+}}V) and clearance of sodium (C\textsubscript{Na\textsuperscript{+}}) were decreased in the Pla2g5−/− group compared with the control group. In accordance, decreased osmolar clearance (C\textsubscript{osm}) was also observed in Pla2g5−/− mice (Fig 3C). On the other hand, FE\textsubscript{Na\textsuperscript{+}} was increased in Pla2g5−/− mice in relation to the WT group (Fig 3D). Thus, besides impairment of tubular
integrity, *Pla2g5<sup>−/−</sup>* mice show changes in tubular function, with a consequent higher FENa<sup>+</sup>. These results suggest that besides maintaining tubular integrity, GV sPLA<sub>2</sub> affects tubular function, such as sodium handling.

**GV sPLA<sub>2</sub> upregulates activity and expression of cortical (Na<sup>+</sup> + K<sup>+</sup>)-ATPase**

The sodium pump (Na<sup>+</sup> + K<sup>+</sup>)-ATPase is one of the principal determinants of tubular sodium transport [29]. Therefore, in this experimental group, we investigated whether the increase in FENa<sup>+</sup> observed in *Pla2g5<sup>−/−</sup>* mice could be correlated with alterations in (Na<sup>+</sup> + K<sup>+</sup>)-ATPase. Activity and α1 subunit expression of (Na<sup>+</sup> + K<sup>+</sup>)-ATPase were evaluated in the renal cortex and medullar preparations of both WT and *Pla2g5<sup>−/−</sup>* mice. Cortical (Na<sup>+</sup> + K<sup>+</sup>)-ATPase
activity was decreased in the \( \text{Pla2g5}^-/^- \) group compared with the control group (Fig 4A). In agreement, cortical expression of the \( \alpha_1 \) subunit of (Na\(^+\) + K\(^+\))-ATPase was decreased in \( \text{Pla2g5}^-/^- \) mice (Fig 4B). However, no significant differences were observed in medullar (Na\(^+\) + K\(^+\))-ATPase activity and \( \alpha_1 \) expression between the \( \text{Pla2g5}^-/^- \) and WT groups (Fig 4C and 4D). These observations of decreased activity and expression of cortical (Na\(^+\) + K\(^+\))-ATPase could explain the increased FENa\(^+\) in \( \text{Pla2g5}^-/^- \) mice. Therefore, GV sPLA\(_2\) is important for tubular function, inducing sodium retention by increasing the activity and expression of cortical (Na\(^+\) + K\(^+\))-ATPase.

**Discussion**

GV sPLA\(_2\) is a highly expressed enzyme in mouse and human heart and placenta [14,18], but GV sPLA\(_2\) mRNA was also detected to a lesser extent in mouse kidney [18]. GV sPLA\(_2\) belongs
to the sPLA₂ family and it is important in phospholipid metabolism and eicosanoid production [4,11]. This enzyme regulates inflammatory processes [10–12,30,31] and is involved in different pathologies [13–16]. However, the role of GV sPLA₂ in renal tissue integrity and function has not been described.

Here, we showed renal expression of GV sPLA₂ and involvement in the maintenance of renal function and sodium handling, revealing new functions for this particular sPLA₂ group. We used mice with targeted disruption of the Pla2g5 gene, and confirmed the absence of Pla2g5 mRNA in Pla2g5⁻/⁻ mice by RT-PCR. Mice lacking GV sPLA₂ expression can be used to address the physiologic role of this enzyme in different tissues [32], because the similar structural and functional features among different sPLA₂ isoenzymes make the development of compounds that selectively inhibit GV sPLA₂ enzymes difficult [1,11,32].

GV sPLA₂ controls, at least in part, the biosynthesis of leukotrienes (LTs) and prostaglandins (PG) derived from membrane phospholipids, but the molecular mechanisms involved and the location of action of sPLA₂ are not totally clear yet [3–7,11,21, 33]. Nevertheless, it has been shown that after being secreted to the extracellular medium, sPLA₂ enzymes hydrolyze phospholipids at the outer cellular surface [33]. In parallel, sPLA₂ enzymes are reinternalized and localized with COX-2 in the perinuclear membrane, ready to promote the conversion of arachidonic acid into eicosanoids [33–39]. LTs and PGs are implicated in many physiologic functions as well as pathologic conditions in different organs, including the kidney [40,41]. Recently, Kvirkvelia et al. [42] showed that PGF₂α promotes cellular recovery of established nephritis in mice, modulating podocyte ultrastructure and foot processes and decreasing proteinuria. In our study, mild glomerular morphologic changes and increased urinary protein excretion were observed in Pla2g5⁻/⁻ mice. These processes are likely linked to a decrease in PGE₂ generation in Pla2g5⁻/⁻ mice. A 50% lower production of PGE₂ and LTC₄ in macrophages as well as reduced COX-2 expression in bone marrow-derived mast cells from Pla2g5-null mice compared with control mice has already been demonstrated [8,21]. In addition, GV

Fig 3. GV sPLA₂ promotes sodium retention. (A) Urinary sodium excretion (U₅₉⁺V), (B) clearance of sodium (C₅₉⁺), (C) osmolar clearance, and (D) F₅Na⁺ in WT and Pla2g5⁻/⁻ mice. The number of mice analyzed is given in Table 1. The results are expressed as means ± SE. *Statistically significant in relation to WT mice (P < 0.05).

doi:10.1371/journal.pone.0147785.g003
sPLA<sub>2</sub> transfection into HEK293 cells induces expression of COX-2, which is the major enzyme involved in the initial conversion of arachidonic acid to prostanoids, such as PGE<sub>2</sub>, in the kidney [43,44].

Although Pla<sub>2g5</sub><sup>−/−</sup> mice showed decreased CCr along with increased urinary protein excretion, there were no profound changes in glomerular structure. However, more prominent tubular changes were observed, suggesting that GV sPLA<sub>2</sub> plays a more pronounced role in the function and integrity of the tubular compartment. Previous studies reported that urinary levels of LDH and γGT are linked to apoptosis of tubular renal cells and, consequently, early diagnosis of kidney disease [45]. Here, we verified intense leakage of LDH and γGT in the urine of Pla<sub>2g5</sub><sup>−/−</sup> mice, which suggests a potential role for GV sPLA<sub>2</sub> in the integrity of tubular cells. In agreement, Murakami et al. [43] showed that GV sPLA<sub>2</sub> is important to cell membrane integrity in HEK293 cells.

Changes in the integrity of tubular cells can induce cell dysfunction, which impairs tubular transport and reabsorption mechanisms, leading to decreased protein reabsorption and proteinuria [26,27,46]. In addition, injury to tubular cells can cause cell dedifferentiation and local inflammation leading to increased renal fibrosis [26,27,40]. Thus, alterations in tubular integrity due to the lack of GV sPLA<sub>2</sub> expression could explain the increased proteinuria and cortical interstitial fibrosis verified in Pla<sub>2g5</sub><sup>−/−</sup> mice. On the other hand, the higher levels of protein in the tubular lumen act in a positive-feedback manner, further promoting apoptosis of tubular cells and interstitial fibrosis [26,47,48].

Fig 4. GV sPLA2 upregulates activity and expression of cortical (Na<sup>+</sup> + K<sup>+</sup>)-ATPase. Expression and activity of (Na<sup>+</sup> + K<sup>+</sup>)-ATPase in WT and Pla<sub>2g5</sub><sup>−/−</sup> mice. ATPase activity from the renal cortex (A) and medulla homogenate (C) was determined by the colorimetric method. Immunoblotting was performed for the (Na<sup>+</sup> + K<sup>+</sup>)-ATPase α1 subunit in (B) the renal cortex and (D) the medullar preparation of both WT and Pla<sub>2g5</sub><sup>−/−</sup> mice, as described in the Materials and Methods (n = 8 per group). The results are expressed as means ± SE. *Statistically significant in relation to WT mice (P < 0.05).

doi:10.1371/journal.pone.0147785.g004
Another indication of the status of tubular integrity and function is the \( \text{FENa}^+ \), which represents the percentage of sodium filtered by the kidney that is excreted in the urine after tubular handling. Therefore, changes in this parameter represent changes in tubular function and damage [28]. The \( \text{FENa}^+ \) was increased in \( \text{Pla2g5}^{-/-} \) mice and this phenomenon was correlated to reduced expression and activity of cortical \((\text{Na}^+ + \text{K}^+)\)-ATPase in these animals. These observations further support the importance of GV sPLA2 in tubular integrity and function.

With regard to tubular sodium handling by \((\text{Na}^+ + \text{K}^+)\)-ATPase activity/expression, Herman et al. [49] observed stimulatory effects of PGE1 and PGE2 on \((\text{Na}^+ + \text{K}^+)\)-ATPase expression/activity in primary cultures of rabbit renal proximal tubule cells. Furthermore, Pöswki et al. [50] showed that PGE2 stimulates the renin-angiotensin-aldosterone system, which stimulates \((\text{Na}^+ + \text{K}^+)\)-ATPase activity, leading to sodium and water retention. Because GV sPLA2 is involved in the generation of PGE2 [11], it is possible that reduced expression/activity of \((\text{Na}^+ + \text{K}^+)\)-ATPase found in \( \text{Pla2g5}^{-/-} \) mice could also be due to reduced PGE2 levels. Moreover, a previous study from our group showed that high concentrations of albumin decreased the expression and activity of \((\text{Na}^+ + \text{K}^+)\)-ATPase in proximal tubule cells [51]. This observation supports the hypothesis that specific tubular alterations in \( \text{Pla2g5}^{-/-} \) mice, including the reduced expression/activity of \((\text{Na}^+ + \text{K}^+)\)-ATPase, are probably due to the increased tubular protein concentration observed in \( \text{Pla2g5}^{-/-} \) mice.

Another protective effect of GV sPLA2 in the kidney could result from its action, through PGE2 production, in promoting resident immune cells with a suppressive phenotype, such as immune inhibitory dendritic cells (DCs) and regulatory Foxp3+ T cells (Tregs). In this regard, Tregs exert protective effects in the kidney, as well as in other organs, against exacerbated and harmful pro-inflammatory responses and acute injury [52–56]. Evidence shows that PGE2 is capable of inducing differentiation of naive T cells into regulatory T cells, and suppressive DCs express high levels of COX-2 along with production of IL-10 and TGF-β, cytokines that are important for differentiation into regulatory Foxp3+ T cells (Tregs) [57–59]. The molecular mechanism involves PLA2 binding, with high affinity, to a mannose receptor (CD206) expressed in DCs and macrophages [60]. Mannose receptor activation upregulates COX-2 expression and increases PGE2 secretion by these cells [58, 59]. In turn, PGE2, via the EP2 receptor in T cells, increases Foxp3 mRNA and protein levels as well as its promoter activity, inducing differentiation of naive T cells into suppressive Foxp3+ T cells (Tregs) [57, 58, 61]. This purported protective effect of PLA2 was confirmed in different models of disease in mice, for instance Parkinson disease and cisplatin-induced nephrotoxicity [58, 59]. In a cisplatin-induced acute kidney injury model, treatment with PLA2 attenuated tissue damage by reducing serum creatinine, blood urea nitrogen, production of pro-inflammatory cytokines, such as IL-6 and TNF-α, and macrophage infiltration [59]. The effects of PLA2 were mediated by the binding and activation of the mannose receptor (CD206) in DCs, followed by an increase in PGE2 secretion. PGE2 induced Treg differentiation and IL-10 production by Tregs and DCs [59]. These IL-10-producing Tregs and DCs exert protective effects in the kidney by reducing monocyte/macrophage infiltration and production of pro-inflammatory cytokines [52–56, 59]. Thus, it is possible that the lack of GV sPLA2 expression, with consequent reduction in local PGE2 production, could decrease the suppressive phenotype of resident immune cells in the kidney, facilitating a prone inflammatory environment and changes in renal tissue homeostasis, such as tubular impairment and fibrosis.

Therefore, despite reports showing renal expression of GV sPLA2 and the physiologic effects of eicosanoids, its enzymatic products, the function of this particular enzyme on the kidney is not well known. Our results highlight a key role of GV sPLA2 in renal homeostasis in the maintenance of tubular cell function and integrity, participating in sodium handling through regulation of cortical \((\text{Na}^+ + \text{K}^+)\)-ATPase expression and activity. Future experiments will further
elucidate the division of labor between GV sPLA₂ and other PLA₂ enzymes as well as the molecular mechanisms involved in the renal effects.

Acknowledgments

The authors would like to thank Mr Vitor Cordeiro Pereira and Mr Mario Luiz da Silva Bandeira (FAPERJ TCT fellowships), Shanserley Leite do Espírito Santo (CNPq fellowship) for the excellent technical support.

Author Contributions

Conceived and designed the experiments: JLSF DBP FMS SSL LSS GMS DZM CMT AASP BLD CCN. Performed the experiments: JLSF DBP FMS SSL LSS GMS DZM. Analyzed the data: JLSF DBP FMS SSL LSS GMS DZM. Contributed reagents/materials/analysis tools: CMT AASP BLD CCN. Wrote the paper: JLSF DBP FMS SSL LSS GMS BLD CCN.

References

1. Dennis EA, Cao J, Hsu YH, Magrioti V, Kokotos G. Phospholipase A2 enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention. Chem. Rev. 2011; 111: 6130–6185. doi: 10.1021/cr200085w PMID: 21910409
2. Burke JE, Dennis EA. Phospholipase A2 structure/function, mechanism, and signaling. J. Lipid Res. 2009; 50 Suppl: S237–S242. doi: 10.1194/jlr.R800033-JLR200 PMID: 19011112
3. Sato H, Taketomi Y, Ushida A, Isogai Y, Kojima T, Hirabayashi T, et al. The adipocyte-inducible secreted phospholipases PLA2G5 and PLA2G2E play distinct roles in obesity. Cell Metab. 2014; 20: 119–132. doi: 10.1016/j.cmet.2014.05.002 PMID: 24910243
4. Murakami M, Sato H, Miki Y, Yamamoto K, Taketomi Y. A new era of secreted phospholipases A2 (sPLA₂). J. Lipid Res. 2015; 56: 1248–1261. doi: 10.1194/jlr.R058123 PMID: 25805806
5. Kikawada E, Bonventre JV, Arm JP. Group V secretory PLA2 regulates TL2-dependent eicosanoid generation in mouse mast cells through amplification of ERK and cPLA2α activation. Blood 2007; 110: 561–567. PMID: 17369491
6. Murakami M, Shimbara S, Kambe T, Kuwata H, Winstead MV, Tischfeld JA, Kudo I. The functions of five distinct mammalian phospholipase A2S in regulating arachidonic acid release. Type Ila and type V secretory phospholipase A2S are functionally redundant and act in concert with cytosolic phospholipase A2: J. Biol. Chem. 1998; 273:14411–14423. PMID: 9603953
7. Han WK, Sapirstein A, Hung CC, Alessandrini A, Bonventr JV. Cross-talk between cytosolic phospholipase A2 alpha (cPLA2 alpha) and secretory phospholipase A2 (sPLA2) in hydrogen peroxide-induced arachidonic acid release in murine mesangial cells: sPLA2 regulates cPLA2 alpha activity that is responsible for arachidonic acid release. J. Biol. Chem. 2003; 278:24153–24163. PMID: 12676927
8. Diaz BL, Satake Y, Kikawada E, Balestrieri B, Arm JP. Group V secretory phospholipase A2 amplifies the induction of cyclooxygenase 2 and delayed prostaglandin D2 generation in mouse bone marrow culture-derived mast cells in a strain-dependent manner. Biochim. Biophys. Acta 2006; 1761: 1489–1497. PMID: 17064958
9. Balsinde J, Shinohara H, Lefkowitz LJ, Johnson CA, Balboa MA, Dennis EA. Group V phospholipase A (2)-dependent induction of cyclooxygenase-2 in macrophages. J. Biol. Chem. 1999; 274: 25967–25970. PMID: 10473537
10. Meilton AY, Muñoz NM, Meilton LN, Birukova AA, Leff AR, Birukov KG. Mechanical induction of group V phospholipase A2(2) causes lung inflammation and acute lung injury. Am. J. Physiol. Lung Cell. Mol. Physiol. 2013; 304: L689–700. doi: 10.1152/ajplung.00047.2013 PMID: 23525785
11. Balestrieri B, Arm JP. Group V sPLA2: classical and novel functions. Biochim. Biophys. Acta 2006; 1761: 1280–1288. PMID: 16945583
12. Ohta S, Imamura M, Xing W, Boyce JA, Balestrieri B. Group V secretory phospholipase A2 is involved in macrophage activation and is sufficient for macrophage effector functions in allergic pulmonary inflammation. J. Immunol. 2013; 190: 5927–5938. doi: 10.4049/jimmunol.1203202 PMID: 23650617
13. Boyanovsky BB, Bailey W, Dixon L, Shridas P, Webb NR. Group V secretory phospholipase A2 enhances the progression of angiotensin II-induced abdominal aortic aneurysms but confers protection against angiotensin II-induced cardiac fibrosis in apoE-deficient mice. Am. J. Pathol. 2012; 181: 1088–1098. doi: 10.1016/j.ajpath.2012.05.037 PMID: 22813854
14. Masuda S, Murakami M, Ishikawa Y, Ishii T, Kudo I. Diverse cellular localizations of secretory phospholipase A2 enzymes in several human tissues. Biochim. Biophys. Acta 2005; 1736: 200–210. PMID: 16188494

15. Kalyvas A, Baskakis C, Magrioli V, Constantiou-Kokotou V, Stephens D, López-Vales R, et al. Differing roles for members of the phospholipase A2 superfamily in experimental autoimmune encephalomyelitis. Brain 2009; 132: 1221–1235. doi: 10.1093/brain/awp002 PMID: 19218359

16. Koduri RS, Gronroos JO, Laine VJ, Le Calvez C, Lambeau G, Nevalainen TJ, Gelb MH. Bactericidal properties of human and murine groups I, II, V, X, and XII secreted phospholipases A2. J. Biol. Chem. 2002; 277: 5849–5857. PMID: 11694541

17. Chen J, Engle SJ, Seilhamer JJ, Tischfield JA. Cloning, expression and partial characterization of a novel rat phospholipase A2. Biochim. Biophys. Acta 1994; 1215: 115–120. PMID: 7947992

18. Valentín E, Ghomashchi F, Gelb MH, Lazdunski M, Lambeau G. On the diversity of secreted phospholipases A(2). Cloning, tissue distribution, and functional expression of two novel mouse group II enzymes. J. Biol. Chem. 1999; 274: 31195–31202. PMID: 10531313

19. Murakami M, Shimbara S, Kambe T, Kuwata H, Winstead MV, Tischfield JA, Kudo I. The functions of five distinct mammalian phospholipase A2S in regulating arachidonic acid release. Type Ila and type V secretory phospholipase A2S are functionally redundant and act in concert with cytosolic phospholipase A2. J. Biol. Chem. 1998; 273: 14411–14423. PMID: 9603953

20. Han WK, Sapirstein A, Hung CC, Alessandri A, Bonventr JV. Cross-talk between cytosolic phospholipase A2 alpha (cPLA2 alpha) and secretory phospholipase A2 (sPLA2) in hydrogen peroxide-induced arachidonic acid release in murine mesangial cells: sPLA2 regulates cPLA2 alpha activity that is responsible for arachidonic acid release. J. Biol. Chem. 2003; 278: 24153–24163. PMID: 12676927

21. Satake Y, Diaz BL, Balestrieri B, Lam BK, Kanaoka Y, Grusby MJ, Arm JP. Role of group V phospholipase A2 in zymosan-induced eicosanoid generation and vascular permeability revealed by targeted gene disruption. J. Biol. Chem. 2004; 279: 16488–16494. PMID: 14761945

22. Degouresse N, Ghomashchi F, Stefanski E, Singer A, Smart BP, Borregaard N, et al. Groups IV, V, and X phospholipases A2s in human neutrophils: role in eicosanoid production and gram-negative bacterial phospholipid hydrolysis. J. Biol. Chem. 2002; 277: 5061–5073. PMID: 11741884

23. Vieyra A, Nachbin L, de Dios-Abad E, GoldFeld M, Meyer-Fernandes JR, Moraes L. Comparison of the functional properties of human and murine groups I, II, V, X, and XII secreted phospholipases A2. J. Biol. Chem. 2005; 280: 4347–4355. PMID: 16040605

24. Martino G, Testa M, Vicinanza P, Habetswallner E. A new method for determination of ATPase activity in tissues homogenates. Boll. Soc. Ital. Biol. Sper. 1978; 54: 2577–2582. PMID: 227435

25. Gorriz JL, Martinez-Castelao A. Proteinuria: detection and role in native renal disease progression. Transplant. Rev. 2012; 26: 3–13.

26. Pinhal CS, Lopes A, Torres DB, Felisbino SL, Rocha Gontijo JA, Boer PA. Time-course morphological and functional disorders of the kidney induced by long-term high-fat diet intake in female rats. Nephrol. Dial. Transplant. 2013; 28: 2464–2476. doi: 10.1093/ndt/gft304 PMID: 24078639

27. Gotfried J, Wiesen J, Raina R Jr Nally JV. Finding the cause of acute kidney injury: which index of fractional excretion is better? Cleve Clin. J. Med. 2012; 79: 121–126. doi: 10.3949/ccjm.79a.11030 PMID: 22301562

28. Moorh JP, Pedersen MP, Buch-Pedersen MJ, Andersen JP, Vilsen B, Palmgren MG, Nissen P. A structural overview of the plasma membrane Na+,K+-ATPase and H+-ATPase ion pumps. Nat. Rev. Mol. Cell. Biol. 2011; 12: 60–70. doi: 10.1038/nrm3031 PMID: 21179061

29. Boyanovsky BB, van der Westhuysen DR, Webb NR. Group V secretory phospholipase A2-modified low density lipoprotein promotes foam cell formation by a SR-A- and CD36-independent process that involves cellular proteoglycans. J. Biol. Chem. 2005; 280: 32746–32752. PMID: 16040605

30. Balestrieri B, Hsu VW, Gilbert H, Leslie CC, Han WK, Bonventr JV, Arm JP. Group V secretory phospholipase A2 translocates to the phagosome after zymosan stimulation of mouse peritoneal macrophages and regulates phagocytosis. J. Biol. Chem. 2006; 281: 6691–6698. PMID: 16407308

31. Belizário JE, Akamini P, Wolf P, Strauss B, Xavier-Neto J. New routes for transgenesis of the mouse. J. Appl. Genet. 2012; 53: 295–315. doi: 10.1007/s13353-012-0096-y PMID: 22569888

32. Balsinde J, Winstead MV, Dennis EA. Phospholipase A(2) regulation of arachidonic acid mobilization. FEBS Lett. 2002; 531: 2–6. PMID: 12401193

33. Murakami M, Nakatani Y, Kudo I. Cellular components that functionally interact with signalling phospholipase A2s. Biochimica et Biophysica Acta (BBA)—Molecular and Cell Biology of Lipids. 2000; 1488: 159–166.

34. Role of GV sPLA2 in Renal Function
Peruchetti D.B., Pinheiro A.A., Landgraf S.S., Wengert M., Takiya C.M., Guggino W.B., Caruso-Neves Kim KP. Mechanism of human group V Phospholipase A2 (PLA2)-induced Leukotriene Biosynthesis in human neutrophils. A POTENTIAL ROLE OF HEPARAN SULFATE BINDING IN PLA2 INTERNALIZATION AND DEGRADATION. J. Biol. Chem. 2000; 276: 11126–11134. PMID: 1118430

Enomoto A, Murakami M, Kudo I. Internalization and degradation of type IIA Phospholipase A2 in mast cells. Biochem. and Biophy. Res. Comm. 2000; 276: 667–672.

Murakami M, Kambe T, Shimbara S, Yamamoto S, Kuwata H, Kudo I. Functional association of type IIA Secretory Phospholipase A2 with the Glycosylphosphatidylinositol-anchored Heparan Sulfate Proteoglycan in the Cyclooxygenase-2-mediated delayed Prostanoid-biosynthetic pathway. J. Biol. Chem. 1999; 274: 29927–29936. PMID: 10514475

Kim YJ, Kim KP, Rhee HJ, Das S, Rafter JD, Oh YS, et al. Internalized group V Secretory Phospholipase A2 acts on the Perinuclear Membranes. J. Biol. Chem. 2000; 277: 9358–9365.

van der Helm HA, Buijtenhuijs P, van den Bosch H. Group IIA and group V secretory phospholipase A2 (2): quantitative analysis of expression and secretion and determination of the localization and routing in rat mesangial cells. Biochim. Biophys. Acta 2001; 1530: 86–96. PMID: 11341961

Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. Science. 2001; 294: 1871–1875. PMID: 11729303

Kvirkvelia N, McMenamin M, Chaudhary K, Bartoli M, Madaio MP. Prostaglandin E2 promotes cellular recovery from established nephrotic serum nephritis in mice, prosurvival, and regenerative effects on glomerular cells. Am. J. Physiol. Renal Physiol. 2013; 304: F463–470. doi: 10.1152/ajprenal.00575.2012 PMID: 23283994

Murakami M, Kambe T, Shimbara S, Higashino K, Hanasaki K, Arita H, et al. Different functional aspects of the group II subfamily (Types IIA and V) and type X secretory phospholipase A2 in regulating arachidonic acid release and prostaglandin generation. Implications of cyclooxygenase-2 induction and phospholipid scramblase-mediated cellular membrane perturbation. J. Biol. Chem. 1999; 274: 31435–31444. PMID: 10531345

Harris RC. Physiologic and pathophysiologic roles of cyclooxygenase-2 in the kidney. Trans. Am. Clin. Climatol. Assoc. 2013; 124: 139–151. PMID: 23874018

Santos C, Marcelino P, Carvalho T, Coelho J, Bispo M, Mourão L, Perdigoto R, Barroso E. The value of tubular enzymes for early detection of acute kidney injury after liver transplantation: an observational study. Transplant. Proc. 2010; 42: 3639–3643. doi: 10.1016/j.transproceed.2010.06.024 PMID: 21094831

Berrut G, Bouhanick B, Fabbri P, Guilloteau G, Bled F, Le Jeune JJ, Fressinaud P, Marre M. Microalbuminuria as a predictor of a drop in glomerular filtration rate in subjects with non-insulin-dependent diabetes mellitus and hypertension. Clin Nephrol. 1997; 48: 92–97. PMID: 9285145

Portella VG, Silva-Filho J.L., Landgraf S.S., de Rico T.B., Vieira M.A., Takiya C.M., Souza M.C., Henriques M.G., Canetti C., Pinheiro A.A., Benjamin C.F., Caruso-Neves C. Sepsis-surviving mice are more susceptible to a secondary kidney insult. Crit. Care Med. 2013; 41: 1056–1068. doi: 10.1097/CCM.0b013e3182746696 PMID: 23385098

Landgraf S.S., Silva L.S., Peruchetti D.B., Sirtoli G.M., Moraes-Santos F., Portella V.G., Silva-Filho J.L., Pinheiro C.S., Abreu T.P., Takiya C.M., Benjamin C.F., Pinheiro A.A., Canetti C., Caruso-Neves C. 5-Lipoxygenase products are involved in renal tubulointerstitial injury induced by albumin overload in proximal tubules in mice. PLoS One 2014; 9: e107549. doi: 10.1371/journal.pone.0107549 PMID: 25302946

Herman M.B., Rajkhowa T., Cutuli F., Springate J.E., Taub M. Regulation of renal proximal tubule Na-K-ATPase by prostaglandins. Am. J. Physiol. Renal Physiol. 2010; 298: F1222–1234. doi: 10.1152/ajprenal.00467.2009 PMID: 20130120

Pöschke A., Kern N., Maruyama T., Pavenstädt H., Narumiya S., Jensen B. L., Nüsing R. M. The PGF2–EP4 receptor is necessary for stimulation of the renin-angiotensin-aldosterone system in response to low dietary salt intake in vivo. Am. J. Physiol. Renal Physiol. 2012; 303: F1435–1442. doi: 10.1152/ajprenal.00512.2011 PMID: 22993066

Peruchetti D.B., Pinheiro A.A., Landgraf S.S., Wengert M., Takiya C.M., Guggino W.B., Caruso-Neves C. (Na+ + K+)-ATPase is a target for phosphoinositide 3-kinase/protein kinase B and protein kinase C pathways triggered by albumin. J. Biol. Chem. 2011; 286: 45041–45047. doi: 10.1074/jbc.M111.260737 PMID: 22057272

Sakaguchi S. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. Nat. Immunol. 2005; 6: 345–352. PMID: 15785760
53. Kinsey GR, Sharma R, Huang L, Li L, Vergis AL, Ye H, et al. Regulatory T cells suppress innate immunity in kidney Ischemia-Reperfusion injury. J. Am. Soc. Nephrol. 2009; 20: 1744–1753. doi:10.1681/ASN.20081111160 PMID:19497969

54. Lee H, Nho D, Chung H-S, Shin M-K, Kim S-H, Bae H. CD4+CD25+ regulatory T cells attenuate cisplatin-induced nephrotoxicity in mice. Kidney Int. 2010; 78: 1100–1109. doi:10.1038/ki.2010.139 PMID: 20463654

55. Ooi JD, Snelgrove SL, Engel DR, Hochheiser K, Ludwig-Portugall I, Nozaki Y, et al. Endogenous foxp3 + t-regulatory cells suppress anti-glomerular basement membrane nephritis. Kidney Int. 2011; 79: 977–986. doi:10.1038/ki.2010.541 PMID: 21248715

56. Kim H, Lee G, Park S, Chung H-S, Lee H, Kim J-Y, et al. Bee venom mitigates Cisplatin-Induced Nephrotoxicity by regulating CD4 + CD25 + Foxp3 + regulatory T cells in mice. Evidence-Based Compl. Alt. Med. 2013: 1–10.

57. Baratelli F, Lin Y, Zhu L, Yang S-C, Heuze-Vourc'h N, Zeng G, et al. Prostaglandin E2 induces FOXP3 gene expression and T regulatory cell function in human CD4+ T cells. J. Immunol. 2005; 175: 1483–1490. PMID: 16034085

58. Chung ES, Lee G, Lee C, Ye M, Chung H-s., Kim H, et al. Bee venom Phospholipase A2, a novel Foxp3+ regulatory T cell Inducer, protects Dopaminergic Neurons by Modulating Neuroinflammatory responses in a mouse model of Parkinson’s disease. J. Immunol. 2015; 195: 4853–4860. doi:10.4049/jimmunol.1500386 PMID: 26453752

59. Kim H, Lee H, Lee G, Jang H, Kim S-S, Yoon H, et al. Phospholipase A2 inhibits cisplatin-induced acute kidney injury by modulating regulatory T cells by the CD206 mannose receptor. Kidney Int. 2015; 88.

60. Mukhopadhyay A, Stahl P. Bee venom Phospholipase A2Is recognized by the Macrophage Mannose receptor. Arch. Biochem. Biophys. 1995; 324: 78–84. PMID: 7503563

61. Sharma S. Tumor Cyclooxygenase-2/prostaglandin E2-Dependent promotion of FOXP3 expression and CD4+CD25+ T regulatory cell activities in lung cancer. Cancer Res. 2005; 65: 5211–5220, PMID: 15958066