

Expression and Localization of Phospholipase D (PLD) in Tubule Cells of Rat Kidney

Expresión y Localización de la Fosfolipasa D (PLD) en las Células Tubulares del Riñón de Rata

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SUMMARY: To establish the basis for understanding the functional significance of phospholipase D (PLD)-mediated signaling in the kidney, the localization of PLD2 was examined in adult rat kidneys. In immunolight microscopy, PLD2-immunoreactivity was weak and diffuse across most intrarenal structures, but was notably stronger in the epithelial cells of collecting ducts, suggesting that the involvement of PLD is higher than in the other tubules. In proximal tubules, distinct immunoreactivity appeared in a wavy line-pattern along the luminal surface. In immunoelectron microscopy, immunogold particles were widely distributed throughout the cytoplasm in collecting ducts. They were deposited on plasma membranes, more frequently on apical membranes, and were also in association with vesicles of varying sizes and in the regions lacking obvious membranous structures. In proximal tubule cells, although immunogold particles were observed in distribution similar to but sparser than in collecting ducts, they were largely absent from apical endosomal vesicle zones and microvilli. Moreover, gold particles were deposited on luminal blebs and within flocculent materials covering the tip of apical microvilli. These materials corresponded to the linear adluminal entities showing distinct immunoreactivity in immunolight microscopy. It is likely that these immunoreactive structures were misinterpreted as PLD2-immunoreactive microvilli in an earlier study by others without ultrastructural confirmation.

KEY WORDS: PLD2; Kidney; Renal tubules; Adluminal substances; Blebs.

INTRODUCTION

Phospholipase D (PLD) is involved in hydrolysis of phosphatidylcholine (PC)--the most abundant membrane phospholipid-- into phosphatidic acid (PA) and choline. The PA can act as a second messenger, affect membrane curvature, or can be hydrolyzed to other lipid second messengers including diacylglycerol (DAG) and lysophosphatidic acid (lysoPA) (Oude Weernink *et al.*, 2007). PLDs are thus involved in various cellular events including secretion, membrane trafficking and cytoskeletal rearrangement as acute events, and proliferation, differentiation, apoptosis and survival of cells as long-term ones (Frohman, 2015).

PLD is composed of two main isoforms--PLD1 and PLD2. While PLD1 exhibits a low basal activity and requires

activators including small guanosine tri-phosphate (GTP) binding proteins (Rho, Arf etc.) and protein kinase C (PKC), PLD2 has a high basal activity and it is less responsive to the activators (Nelson & Frohman, 2015). Isoforms are generally supposed to be localized in appropriate regions of cells to produce diverse structural and functional pools of a given protein molecule and this is also the case for PLD. Studies of cultured cells, when transfected with cDNAs for the PLD isoforms, have demonstrated that exogenous PLD1 is localized mainly in various intracellular membranes, and PLD2 in the plasma membrane (Liscovitch *et al.*, 2000; Freyberg *et al.*, 2002; Sarri *et al.*, 2003). However, in vitro studies using overexpressed exogenous protein molecules in cultured cells may not reflect the situation in vivo, and the intracellular distribution of PLD is still controversial.

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There is still little information on the localization of endogenous PLD in cells *in situ*, although such information is critical for eventual understanding of its functional significance. The paucity of information may be because the expression levels of PLD in cells *in situ* are relatively low and because few antibodies appropriate for immunohistochemical analyses are available for PLD isoforms. We have reported detailed localization of the PLD isoforms at protein levels in the salivary glands and at mRNA levels in the brain of developing and adult rodents (Saito *et al.*, 2000; Khongyut *et al.*, 2019, 2023).

In view of the information described above, the present study was undertaken to examine the expression and localization of PLD in the kidney *in situ* of young adult rats in light and electron microscopy and Western blotting by using the same antibodies that were employed in our previous studies cited above. The kidney was selected because there have been only a limited number of studies that reported on PLD-localization in normal kidney *in situ*, but discrepancies remain to be clarified about exact localization (Bosch *et al.*, 2001; Yang *et al.*, 2005; Kim *et al.*, 2007).

MATERIAL AND METHOD

Animal preparation

Ten male Sprague-Dawley rats, aged 8 weeks (200 g; purchased from Siam Nomura Laboratory Animal Center, Co., Ltd., Bangkok, Thailand), were housed under standard vivarium conditions and used in the present experiments. Following anesthesia, rats were transcardially perfused with 100 mL of physiological saline, followed by 100 mL of 4 % paraformaldehyde in 0.1 M phosphate buffer (PB). The kidneys were excised and post-fixed overnight in the same fixative. Specimens were subsequently immersed in 30 % sucrose in PB for cryoprotection until processed for immunohistochemical analysis.

Western blotting analysis

For Western blot analysis, the kidneys excised after perfusion with physiological saline were homogenized in lysis buffer. Total protein concentrations were determined, and 20 µg aliquots were boiled for 10 min in 2× SDS sample buffer before separation by SDS-PAGE (12.5 %). Proteins were transferred electrophoretically onto PVDF membranes (Immobilon-P, Merck, Kenilworth, NJ). Membranes were blocked with 5 % skim milk (wt/vol) in TBS containing 0.1 % Tween-20 (TBST) and incubated overnight at 4 °C with rabbit polyclonal antibodies against peptides corresponding to human PLD1 (Ab224) and PLD2 (Ab-58) at 0.1 µg/mL in 5 % skim milk/TBST. The synthesis and specificity of

these antibodies have been previously described (Banno *et al.*, 1999; Zhao *et al.*, 2000).

Immunolight microscopy examination

For immunodetection by DAB (diaminobenzidine) light microscopy, 20-µm-thick cryosections were prepared from fixed, sucrose-embedded specimens using a cryostat. Sections mounted on glass slides were incubated with 0.3 % H₂O₂ in methanol for 10 min, followed by blocking with 10 % normal goat serum in PBS for 30 min. Sections were then incubated overnight at room temperature with rabbit polyclonal IgG antibody against PLD2 at a final concentration of 1 µg/mL in 0.1 % Triton-X/PBS. After washing, sections were incubated for 1 h at room temperature with biotinylated anti-rabbit IgG secondary antibody (Abcam, Cambridge, MA, USA), and immunoreactive sites were visualized using the ABC kit (Vector Laboratories, Burlingame, CA, USA). To identify immunoreactive profiles as proximal tubules or collecting ducts, pairs of serial sections were mounted on separate slides, with one stained for PLDs and the other for AQP1 or AQP2 (Cat# AB2219 and Cat# 178612, Merck Millipore, Billerica, MA, USA). As an antibody for NCC was not available for this study, distal tubules were identified by immunogold electron microscopy based on characteristic basal infoldings and sparse apical microvilli in normal kidneys.

Immunogold electron microscopy examination

For pre-embedding immunogold electron microscopy, cryostat sections placed on poly-L-lysine-coated plastic slides were pretreated with 0.1 % saponin and subsequently incubated with the PLD2 antibody. Sections were then reacted with goat anti-rabbit IgG (Cat# 2003; Nanoprobes, Yaphank, NY) conjugated to ultra-small gold particles (1:100 dilution), followed by silver enhancement using the Aurion R-GENT SE-LM kit (Cat# 500.01; Aurion, Hatfield, PA). Ultrathin sections were stained with uranyl acetate and lead citrate and examined using a JEM1010 transmission electron microscope (JEOL, Tokyo, Japan).

RESULTS

Western blotting results

A distinct band of approximately 106 kDa, corresponding to PLD2, was observed in kidney homogenates from male rats under normal conditions (Fig. 1). In contrast, the band corresponding to PLD1 (approximately 120 kDa) was barely detectable in kidney homogenates from rats under normal conditions (data not shown). Therefore, subsequent immunohistochemical analyses focused exclusively on PLD2.

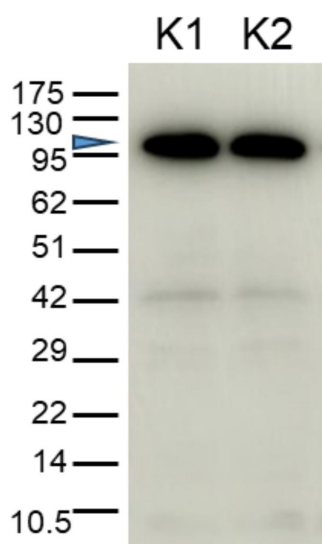
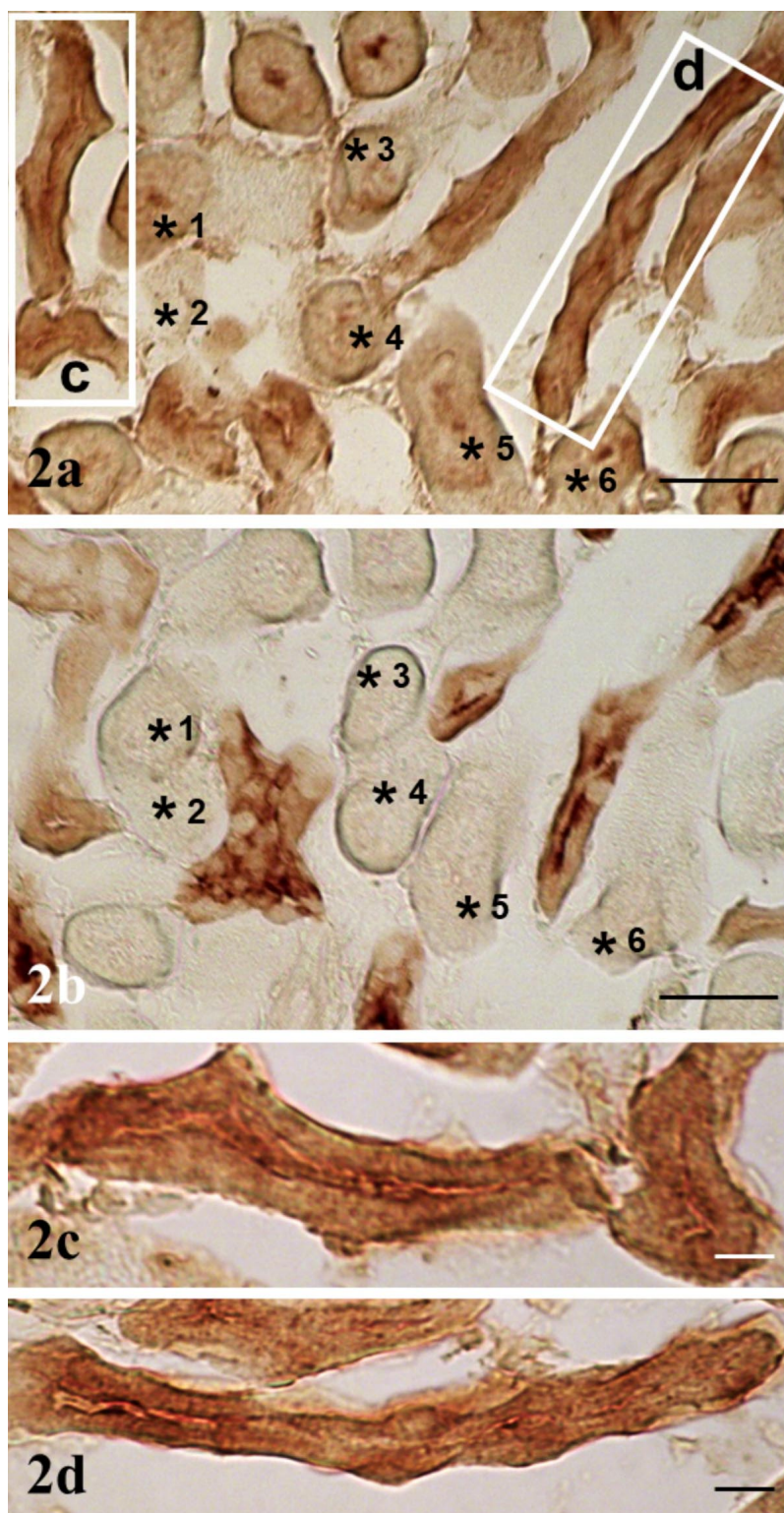


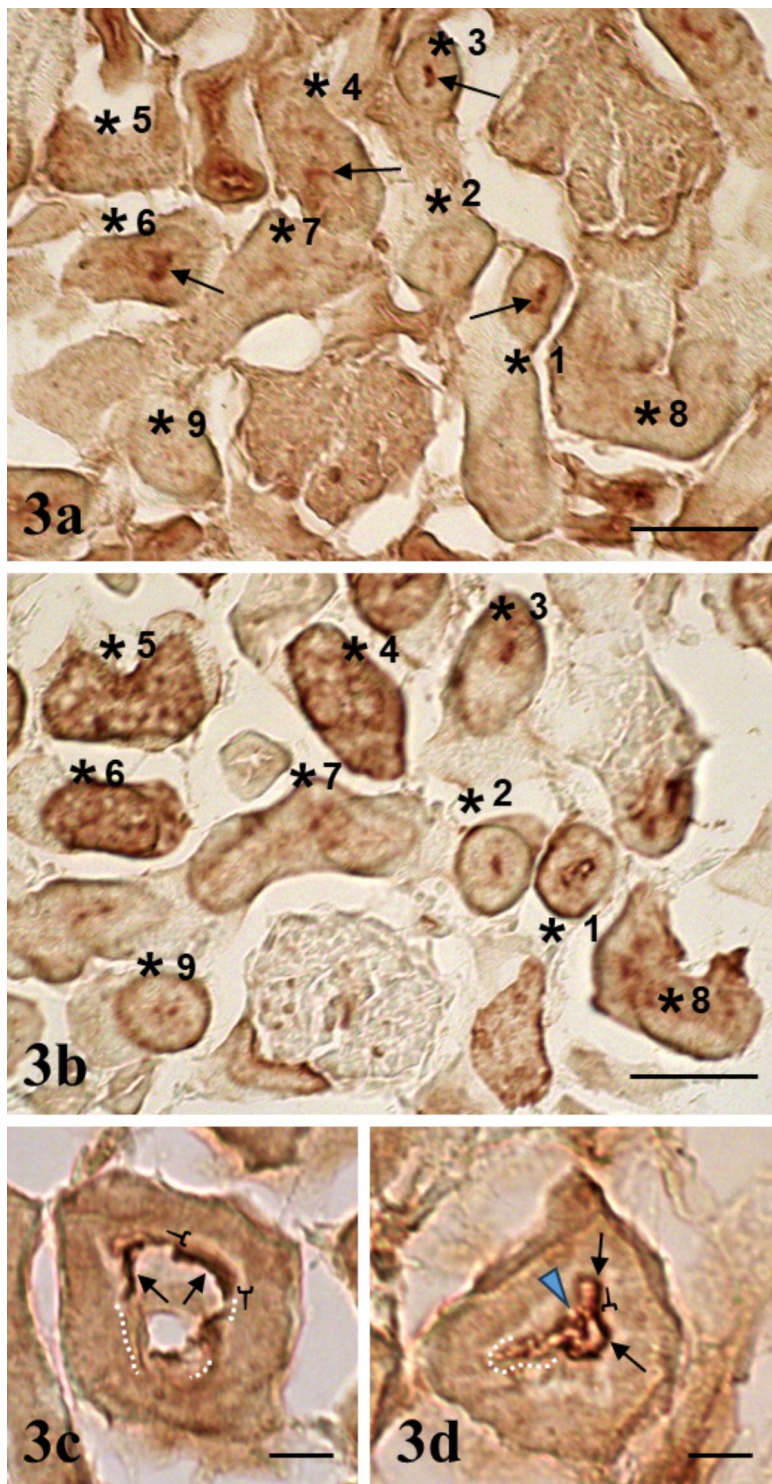
Fig 1. Western blotting of normal rat kidney homogenates (K1 & K2) showing the distinct bands (MW: 106 kDa) for PLD2.

Immunolight microscopy results

PLD2-immunoreactivity was diffusely weak in nearly all structures of the normal renal cortex, including tubules, ducts, glomeruli, and capillaries. Among these structures, relatively few ducts---thinner epithelial walls and diameters---showed markedly higher PLD2 immunoreactivity, which was distributed throughout their epithelial cells. Immunostaining of serial sections for PLD2 and AQP2 revealed that the ducts with stronger PLD2-immunoreactivity were AQP2-positive collecting ducts. Distinct immunoreactivity was observed along the lumens of some ducts (Figs. 2a-d). On the other hand, in proximal tubules identified by positive immunoreaction for AQP1, increased immunoreactivity appeared as a dot- or irregular line-form in the center of many tubules whose epithelial cells otherwise exhibited weak and diffuse labeling. In proximal tubules showing more or less wide lumens, such increased immunoreactivity appeared as wavy lines delineating the luminal contours. These immunoreactive lines were frequently discontinuous and did not extend along the entire luminal contour. At times, they formed small, partial convex projections into luminal spaces (Figs. 3a-d).



Figs. 2a-d. Immunolight micrographs of serial sections stained for PLD2 (2a) and collecting duct marker AQP2 (2b). Higher magnifications of boxed areas in 2a are shown in 2c and 2d. PLD2 immunoreactivity is strong and diffuse in AQP2-positive collecting ducts, but weak in AQP2-negative tubules. Numbered asterisks in 2a and 2b correspond. Bars, 50 µm.



Figs. 3a-d. Immunolight micrographs of serial sections immunostained for PLD2 (3a) and for proximal tubule marker AQP1 (3b). Higher magnifications of selected tubules are shown in 3c and 3d. PLD2 immunoreactivity appeared as central rings or wavy lines (arrows), which do not match typical brush margins because of irregular thickness and partial lack along luminal contours (white broken lines). Blebs are highlighted by an arrowhead. Numbered asterisks correspond to 3a and 3b. Bars represent 50 μ m.

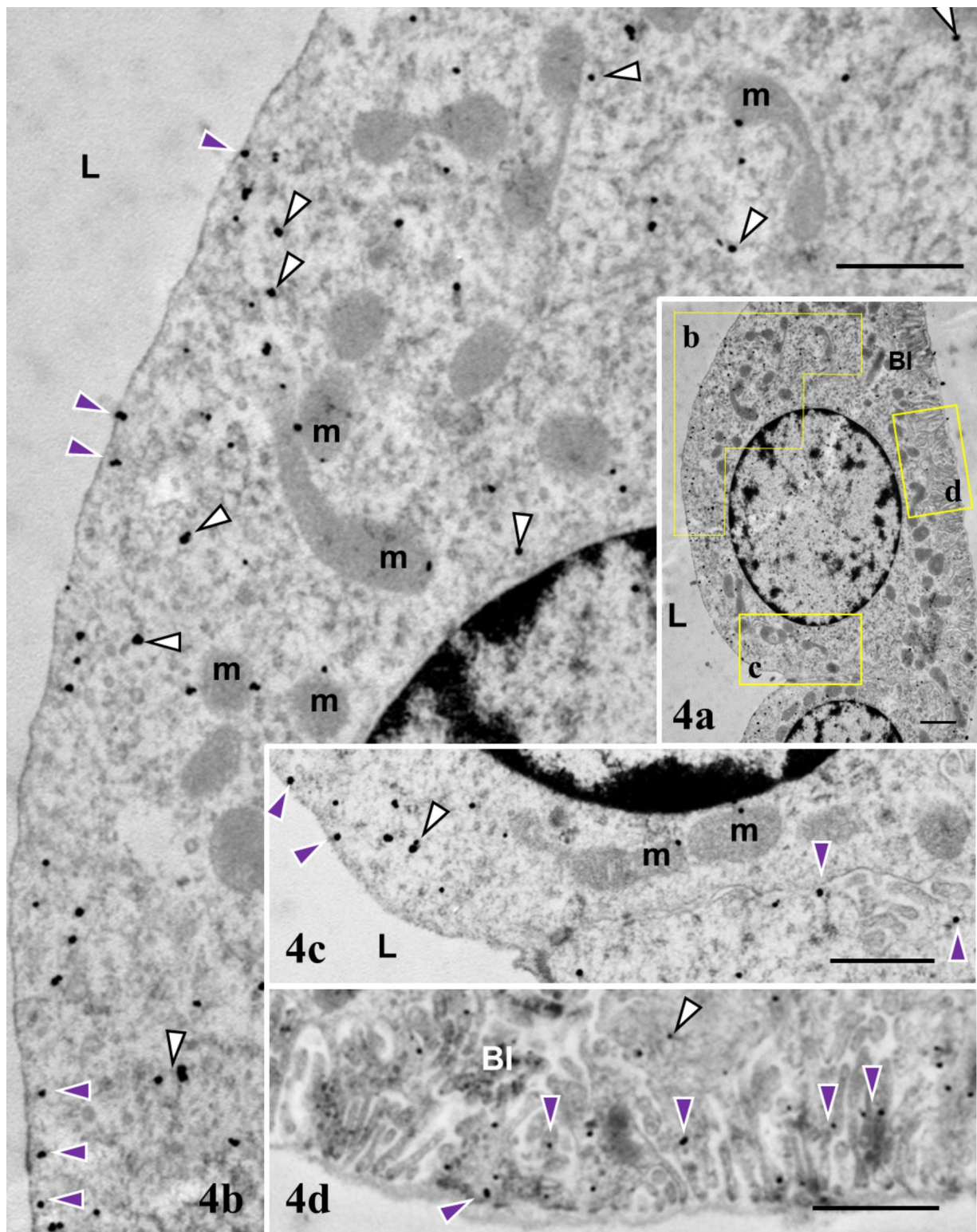
Since all ductal structures showing strong and diffuse PLD2-immunoreactivity were confirmed to be collecting ducts, distal tubules were likely included among the tubular structures showing weak and diffuse immunoreactivity, together with proximal tubules. The identification of distal tubules with weak and diffuse immunoreactivity was further clarified in the subsequent immunoelectron microscopy.

In control experiments, omitting the primary antibody, no immunoreactions were observed in any portions of kidney sections of rats (data not shown).

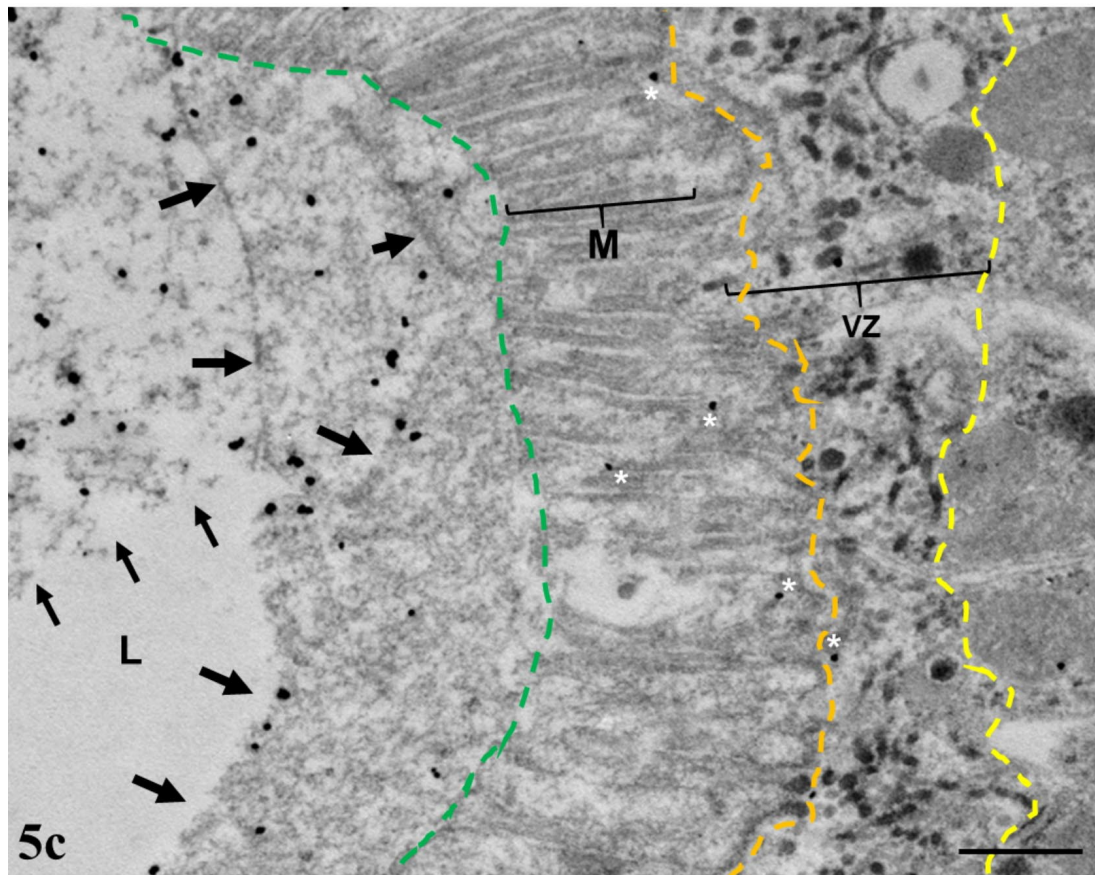
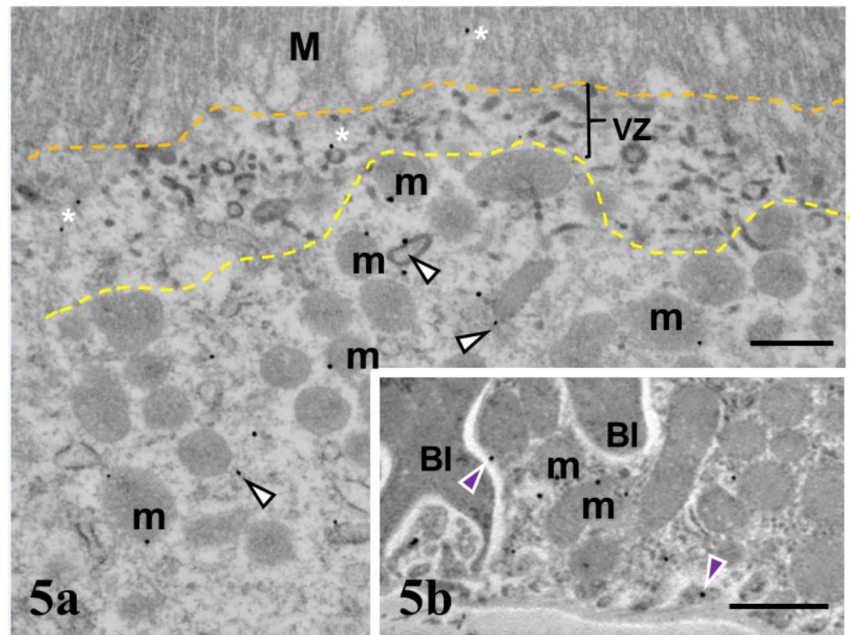
Immunonanogold electron microscopy results

In collecting ducts characterized by short and thin basal foldings without mitochondrial association, immunogold particles were sparsely dispersed throughout the entire cytoplasm. Gold particles were frequently deposited on the apical plasma membranes, and less frequently on the lateral and basal membranes, as well as on intracellular vesicles and vacuoles, and occasionally along the contours of mitochondria. Vesicles and vacuoles without association with particles were also present. Some particles were found in the cytoplasm without any apparent association with membranous organelles and cytoskeletal structures (Figs. 4a-d).

In proximal tubules, immunogold particles were similarly but more sparsely observed throughout the entire cytoplasm. Some particles were associated with vesicles, vacuoles, and occasionally with the contour of mitochondria, while others were not closely related to any membranous and filamentous structures (Fig. 5a). Few particles were seen along the lateral and basal plasma membranes (Fig. 5b). To be noted, immunogold particles were largely absent from apical microvilli and the underlying endosomal vesicle zones. In contrast, numerous immunogold particles were observed in adluminal extracellular regions of varying thickness. These regions were occupied by flocculent materials of different compactness/electron density which were



Figs. 4a-d. Immunogold electron micrographs of collecting ducts with basal infoldings (BI). Higher magnifications of boxed regions in 4a are shown in 4b-d. Immunogold particles are mainly on apical plasma membranes (purple arrowheads: 4b, 4c), also seen on lateral/basal membranes (purple arrowheads: 4c, 4d), vesicles/vacuoles (white arrowheads), and mitochondria (m), as well as the cytoplasmic matrix without apparent association with membranous or filamentous structures. No particles are present in the tubule lumens. Bars, 1 μ m (4a, 4d); 600 nm (4b, 4c).



Figs. 5a-c. Immunogold electron micrographs of proximal tubules with mitochondria (m)-associated basal infoldings (BI), apical microvilli (M), and endosomal vesicle zones (VZ). The apical (5a) and basal (5b) domains are shown. Immunogold particles are dispersed throughout the cytoplasm, but largely absent in microvilli and endosomal vesicle zones (VZ). Fig 5c shows highlights adluminal flocculent regions rich in immunogold particles, having variable contours (arrows) and in contiguity with microvillous tips. White asterisks indicate a few gold particles. L: tubule lumen. Bars, 600 nm (5a, 5c); 1 μ m (5b).

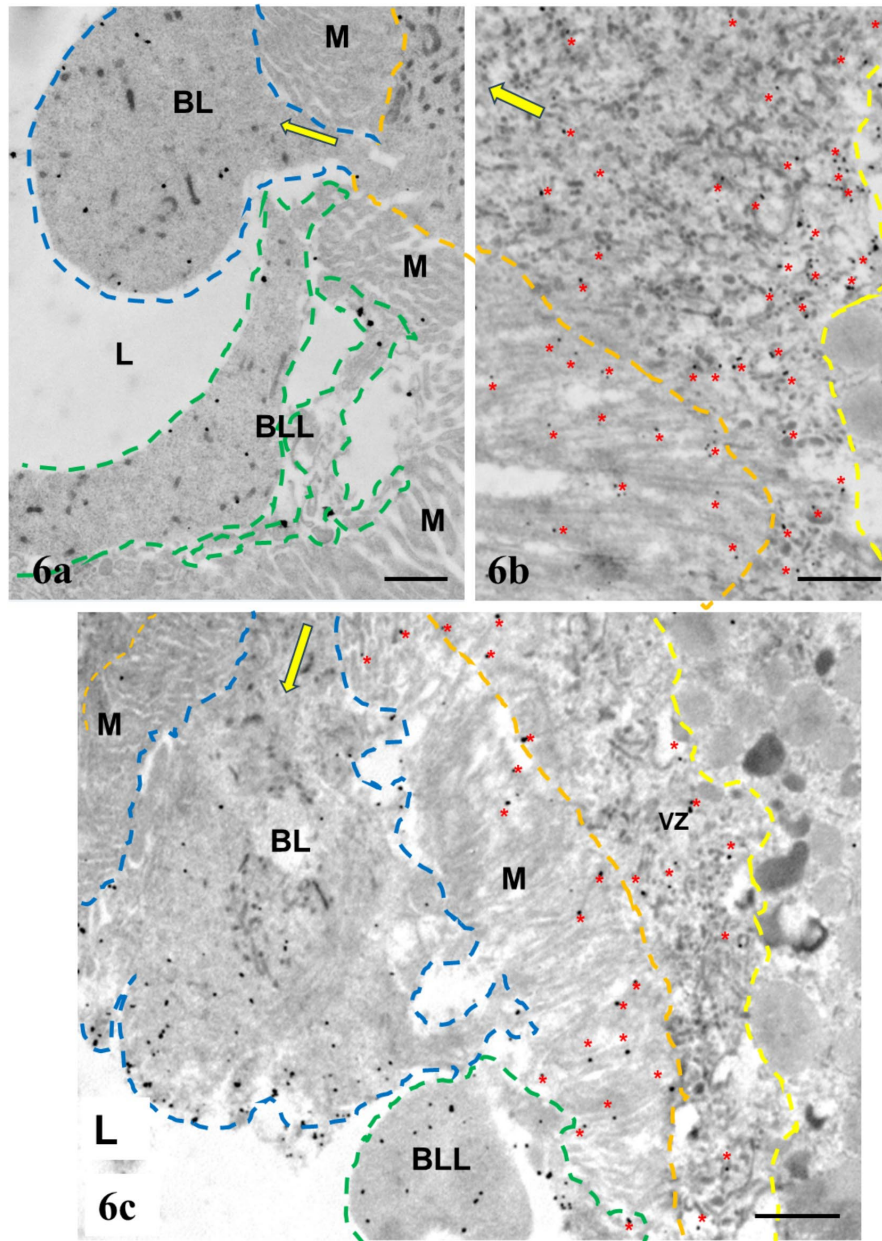
contiguous with, or covered, the tips of microvilli. This distribution contrasted with the complete absence of gold particles in luminal empty spaces that lacked any material contents (Fig. 5c). While the margin of such flocculent materials against the empty lumen was largely smooth in such a way as appearing enclosed by membranous delineations but largely without clear recognition of

membranous structures, peripheries of some flocculent materials were irregularly shaped with an appearance of dispersion of proteinaceous material.

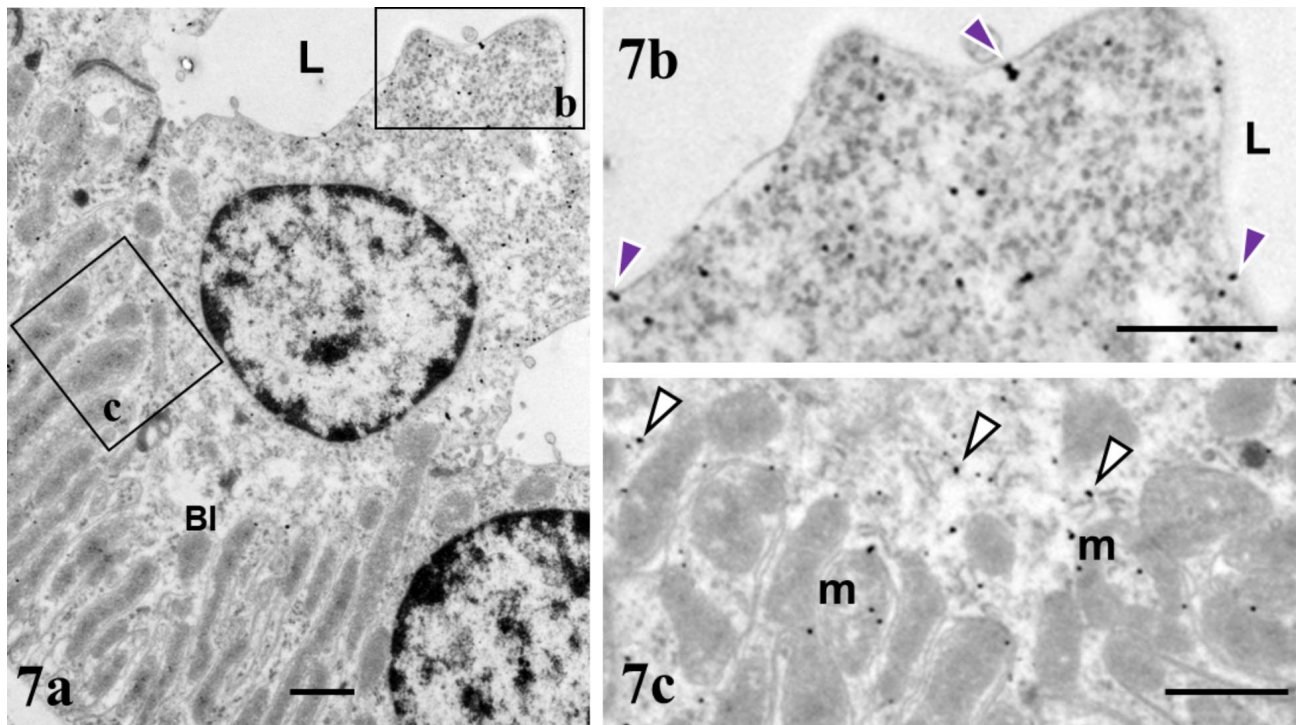
On the other hand, immunogold particles were observed randomly within luminal blebs that are bulged from the apical cellular domain in proximal tubules. The blebs

corresponded to small, partial convex projections small, partial convex projections of immunoreactive lines along the luminal contours, which were observed in immunolight microscopy. In adluminal regions near the bleb connections with the apical cytoplasm, structurally similar entities---smaller and more irregular or flattened in shape than typical blebs--- were frequently observed. These structures were often found in close apposition to, or covering, the tip of microvilli of tubule cells, and they contained randomly distributed immunogold particles. Some of the structural entities were fragmented. To be noted in these regions, a substantial number of immunogold particles were associated with vesicles and tubules within endosomal vesicle zones and were also present on some microvilli. This occurrence of particles differed from their absence in most of these apical structural specializations described above (Figs. 6a-c). Additionally, portions of the vesicle zone containing immunogold particles appeared to extend into the bases of blebs.

In distal tubules, identified by characteristic basal infoldings associated with elongated mitochondria, the distribution pattern of immunogold particles was essentially similar to, but more sparsely than, that observed in collecting ducts (Figs. 7a-c). Particles were found on apical membranes, vesicles and some mitochondria, though some particles were also observed throughout the cytoplasm without clear association with cellular organelles (Fig. 7b).



Figs. 6a-c. Immunogold electron micrographs of proximal tubule apical domains and adluminal regions near luminal blebs (BL). Flocculent material regions of varying thickness (BLL, bleb-like, green broken lines) contain numerous immunogold particles and cover the tips of microvilli (M). Near bleb bases, immunogold particles are present (red asterisks) in association with endosomal vesicles, microvilli, and inside blebs and bleb-like entities. Thick yellow arrows indicate projection of apical domains including vesicle zones (VZ) together with particles into blebs. Bars, 600 nm (6a); 1 mm (6b, 6c).



Figs. 7a-c. Immunogold electron micrographs of distal tubules with mitochondria-associated basal infoldings (BI) and apical sparse microvilli. Higher magnifications of the boxed areas in 7a are shown in 7b and 7c. Immunogold particles are dispersed throughout the entire cytoplasm, mainly along the plasma membrane (purple arrowheads) and on vesicles/vacuoles (white arrowheads) m; mitochondria with particles. Bars, 1 µm.

DISCUSSION AND CONCLUSION

The detection of a band for PLD 2 and absence of PLD1 expression in rat kidneys by the present Western blot analysis were consistent with findings previously reported in human renal cell lines *in vitro* using the same antibodies as those used in this study (Banno *et al.*, 1999; Zhao *et al.*, 2000). This finding supports the validity of the immunoreactions observed in the immunohistochemical analysis. The specificity of the immunoreaction was further supported by the absence of immunoreactivity when the primary antibody was omitted. The lack of PLD1 expression in homogenates of rat kidneys has also been reported previously by Min *et al.* (2001), though the detection of PLD1b transcript by RT-PCR and of PLD1 protein by Western blotting has been reported in primary cultures of rabbit collecting duct cells (Bosch *et al.*, 2001).

Two major findings were obtained from the present immune-light and electron microscopic analyses. The first, PLD2-immunoreactivity was stronger in collecting duct cells than in other renal structures, including renal tubules, glomeruli and blood vessels. Second, PLD2-immunoreactivity was largely absent in apical microvilli and subjacent endocytosis vesicle zone in proximal tubule cells. Instead, it was revealed to occur in materials located in

contiguity with, or covering, the tips of microvilli as well as intraluminal blebs.

Regarding the first major finding of collecting ducts, the wide localization of immunogold particles observed by electron microscopy was consistent with the diffuse PLD2-immunostaining of ductal cells detected by immunolight microscopy. The rare occurrence of gold particles in the nuclei and in the ductal empty lumen of any tubules/ducts supports the validity of particle localization within the cells. Their presence on the apical plasma membranes was not as concentrated as might be expected based on previous reports indicating that exogenous PLD2 is localized mainly to the plasma membrane of cultured cells, as cited in this Introduction. Considering the difference in thickness between ultrathin sections used for electron microscopy and regular sections for light microscopy, the ultrastructural presence of particles on the apical membranes appears to correspond to the distinct immunostaining along the luminal surface observed in light microscopy.

The presence of PLD2 transcript has been reported in cultured cells derived from rabbit collecting duct cells by RT-PCR analysis (Bosch *et al.*, 2001). In their study, PLD

activity in these duct cells was stimulated by exogenous vasopressin via its receptor localized in the ductal cells. The authors suggested that PLD functions as a key intracellular signaling enzyme in collecting duct cells, translating vasopressin signaling into regulatory actions on Ca^{2+} and sodium transport. Since PLD2-immunoreactivity was stronger in collecting duct cells of rats in the present study, further pharmacological studies are required to examine whether participation of PLD in vasopressin signaling is also the case in rat collecting ducts.

Regarding the intracellular localization of PLD2 in collecting ducts, immunogold labeling was observed not only along the plasma membrane but also on vesicles of varying sizes and in regions lacking obvious membranous or filamentous structures throughout the cells. This distribution is noteworthy because PLD2 has generally been reported to localize primarily to the plasma membrane, where its major substrate, phosphatidylcholine (PC), is abundant. In contrast to PLD1, PLD2 is considered to be constitutively associated with the plasma membrane, although it can be internalized into early endosomes following stimulation by certain factors (Hodgkin *et al.*, 2000; Bowling *et al.*, 2021). Most previous information on PLD2 localization has been derived from studies using culture cells artificially transfected with exogenous PLD2 constructs. However, in the present *in situ* analysis of collecting duct cells, PLD2-immunoreactivity marked by gold particles was detected not only along the plasma membranes but also on some vesicles and vacuoles, as well as at sites not clearly associated with identifiable structural components. These findings suggest that endogenous PLD2 in native cells may exhibit a broader intracellular distribution than that described in transfected cell systems.

One possible explanation for this diverse localization is that intrinsic PLD2 does not remain exclusively associated with the plasma membrane but can translocate between soluble and particulate fractions under certain conditions, including stimulatory states. Supporting this interpretation, PLD has been reported by immunoblotting to exist in both particulate and soluble fractions of primary cultured proximal tubule cells of rats, although it is predominant in the particulate fraction (Santiago *et al.*, 2007). Such dynamic intracellular distribution of PLD2 may also account for the ultrastructural localization observed in proximal and distal tubule cells in the present study.

The second major finding---the rare occurrence of PLD2-immunogold particles in the apical endosomal vesicle zones and microvilli of proximal tubules---, was somewhat unexpected, given the known functional significance of PLD in relation to roles played by these two cellular

specializations. The apical endosomal apparatus plays a critical role in regulating the composition of glomerular filtrate composition by controlling the turnover of bioactive molecules through active endocytosis (Negri, 2006). Membrane curvature required for vesicle fission during endocytosis is facilitated by phosphatidic acid (PA), the major product generated by PLD. In addition, PLD has been implicated in several processes relevant to the maintenance and function of renal brush margin microvilli, including actin regulation, vesicle trafficking, and apical membrane growth (Jenkins & Frohman, 2005; Oude Weernink *et al.*, 2007). However, direct experimental evidence supporting these proposed signaling roles in proximal tubule brush margin membranes remains limited.

In this context, the immunolight microscopic finding reported by Yang *et al.* (2005), warrant attention. In their study of mouse kidney, PLD2-immunoreactivity appeared as a ring- or wavy line-pattern along the luminal surface of proximal tubules, which they interpreted as PLD2 localization in the brush margin membrane. However, the luminal surface staining pattern they observed is likely comparable to the adluminal immunoreactive lines identified in the present immunolight and electron microscopic analyses. In this regard, the immunolight microscopic feature of the immunoreactive lines along the tubule lumen is noteworthy: their frequent discontinuity along the entire luminal contour and their small, partial convex projections into luminal spaces. If the immunoreactive lines truly correspond to the brush margins as they interpreted, these features are not compatible with the constant existence of brush margins in normal proximal tubules. Thus, their conclusion of PLD2 localization within the brush-margin membranes---including microvilli and the subjacent vesicle zone---may be premature, as it lacked ultrastructural confirmation. The implication of PLD2 in the regulatory function of apical membrane specializations requires renewed evaluation. It is because the current immunogold electron microscopic evidence indicates no significant PLD2-immunoreactivity in the main portions of apical microvilli and adjacent vesicle zones.

One renewed consideration is that PLD2, diversely localized within the cytoplasm beneath the endosomal vesicle zone in proximal tubule cells, may be rapidly recruited to microvillous membranes and the endosomal apparatus when required. This point aligns with the functional significance of the localization pattern of immunogold particles throughout the cytoplasm of collecting duct cells. Another important point is that PLD2 levels below the histochemical detection threshold may still be sufficient for its regular role in maintenance of microvillous structures and supporting endocytic activity within the endosomal apparatus. Thus,

when interpreting immunohistochemical data, it is crucial to remember that positive immunoreactivity reflects antigen levels above the detection threshold, while PLD is in fact ubiquitous across all cell types (Santiago *et al.*, 2007).

Regarding immunogold precipitation, attention should also be given to an exception: A substantial number of immunogold particles were detected in regions of two apical structures located near the bases of luminal blebs, in contrast to lack of particles in most parts of the regions. Luminal blebs in renal proximal tubules are cytoplasmic protrusions from the apical surface. Although the blebs were sometimes regarded as a key marker of ischemia-induced injury, a recent study by Huang *et al.* (2023), has disclosed that these structures, termed extracellular vesicles in that paper, represent an intrinsic physiological function of normal proximal tubule cells and serve as self-renewal of their endogenous organelles and cytoplasmic contents. They reported that these structures were a balloon-like fraction of cytoplasm with an average diameter of ~5mm and were exclusively squeezed through the dense microvilli and extruded into the extracellular lumen space. The present observation of blebs is consistent with their description. According to the cell biological information, formation of blebs/extracellular vesicles results from the breakdown of filamentous actin, which normally supports the microvilli, thereby allowing the apical membrane to bulge into the tubule lumen, in which PLD is implicated through PA (Mahankali *et al.*, 2011). In these exceptional regions of the apical special structures, portions of the endosomal vesicle zone containing immunogold particles appeared to extend into the bases of the blebs. These findings suggest that bleb formation requires PLD2 at levels higher than those needed for routine maintenance of microvilli and the endosomal apparatus, and that PLD2 is additionally recruited from the surrounding cytoplasm at these sites.

What is the significance of PLD2-immunoreactive materials localized adluminally above the tip of apical microvilli in proximal tubule cells? One issue to be considered is the possibility that the contents of blebs, when their enclosing membranes rupture for reasons that remain unclear, may be retained on the microvillus tips. This could correspond to the distinct immunoreactive materials observed on the adluminal surface of tubules by immunolight microscopy in the present study as well as to that by Yang *et al.* (2005). Another issue to be considered is that the substances deposited along the adluminal surface represent PLD2-like immunoreactive material rather than authentic PLD2, although no significant immunostaining was detected in control sections incubated with non-immune serum or with several antibodies unrelated to PLD (data not shown). Additionally, even if blebs and the extracellular materials

supposedly derived from ruptured blebs contain authentic PLD2, its functional significance within tubular lumens remains an enigma. Further studies using kidneys from PLD2 gene-knockout rats, if available, will be required to clarify this issue.

Taken all together, this is the first report about ultrastructural localization of PLD2 in three renal tubules/ducts. Based on these findings, further molecular pharmacological studies on the functional significance of PLD, particularly PLD2, in renal tubules/ducts are expected in the near future.

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Compliance with Ethical Standards. All experimental procedures were performed in strict accordance with the Guidelines for the Care and Use of Laboratory Animals at Khon Kaen University. The study protocol was reviewed and approved by the Animal Ethics Committee of Khon Kaen University, following the Ethics of Animal Experimentation of the National Research Council of Thailand (Reference IACUC-KKU-16/68).

Conflicts of interest. The authors declare that there are no conflicts of interest.

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RESUMEN: Para establecer las bases que permitan comprender la importancia funcional de la señalización mediada por la fosfolipasa D (PLD) en el riñón, se examinó la localización de PLD2 en riñones de ratas adultas. Mediante microscopía inmunoluminiscente, la inmunorreactividad de PLD2 fue débil y difusa en la mayoría de las estructuras intrarrenales, pero notablemente más intensa en las células epiteliales de los conductos colectores, lo que sugiere que la participación de PLD es mayor que en los demás túbulos. En los túbulos proximales, se observó una inmunorreactividad distintiva en un patrón lineal ondulado a lo largo de la superficie luminal. En la inmunomicroscopía electrónica, las partículas de inmunoro se distribuyeron ampliamente por todo el citoplasma de los conductos colectores. Se depositaron en las membranas plasmáticas, con mayor frecuencia

en las membranas apicales, y también se asociaron con vesículas de diversos tamaños y en regiones sin estructuras membranosas evidentes. En las células de los túbulos proximales, si bien se observó una distribución de partículas de inmunoro similar, aunque más dispersa a la de los conductos colectores, estas estuvieron prácticamente ausentes en las zonas de vesículas endosomales apicales y en las microvellosidades. Además, se depositaron partículas de inmunoro en las protuberancias lumbinales y dentro de materiales floculantes que cubrían la punta de las microvellosidades apicales. Estos materiales correspondían a las entidades adlumbinales lineales que mostraban una inmunorreactividad distintiva en la inmunomicroscopía óptica. Es probable que estas estructuras inmunorreactivas se hayan interpretado erróneamente como microvellosidades inmunorreactivas a PLD2 en un estudio anterior realizado por otros autores sin confirmación ultraestructural.

PALABRAS CLAVE: PLD2; Riñón; Túbulos renales; Sustancias adlumbinales; Ampollas.

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