Annals of Anatomy 222 (2019) 21–27

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.com/locate/aanat

RESEARCH ARTICLE

Expression and localization of VIAAT in distal uriniferous tubular epithelium of mouse

Waraporn Sakaew[a], Apussara Tachow[b], Wipawee Thoungseabyoun[c], Suthankamon Khrongyut[a], Atsara Rawangwong[a], Yada Polsan[a], Watanabe Masahiko[d], Hisatake Kondo[a,e], Wiphawi Hipkaeo[a,*]

[a] Electron Microscopy Unit, Department of Anatomy, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand
[b] Mahidol University, Nakhonsawan Campus, Nakhonsawan 60130, Thailand
[c] Faculty of Medicine, Siam University, 38 Phet Kasem Road, Bang Wa, Phasi Charoen, Bangkok 10160 Thailand
[d] Department of Anatomy, Graduate School of Medicine, Hokkaido University, Sapporo, Japan
[e] Department of Anatomy, Graduate School of Medicine, Tohoku University, Sendai, Japan

ARTICLE INFO

Article history:
Received 25 December 2017
Received in revised form 28 October 2018
Accepted 1 November 2018

Keywords:
VIAAT
Distal uriniferous tubule
Plasma membrane
Kidney
Mouse

ABSTRACT

Vesicular inhibitory amino acid transporter (VIAAT) is a transmembrane transporter which is responsible for the storage of gamma-aminobutyric acid (GABA) or glycine in synaptic vesicles. According to recent studies, GABA is known to be expressed in the kidney. For clear understanding of the intra-renal GABA signaling, the localization of VIAAT was examined in the present study. Intense immunoreactivity was found largely confined to the distal tubule epithelia, especially distinct in the inner medulla, although the immunoreactivity was discerned more or less in all tubules and glomeruli. No distinct immunoreactivity was seen in capillary endothelia or interstitial fibroblasts. In immuno-DAB and immuno-gold electron microscopy, the immunoreaction was found at the basal infoldings of plasma membranes and basal portions of the lateral plasma membranes, but not in any vesicles or vacuoles within the distal tubular cells. The significance of the enigmatic finding, localization of a vesicular molecule on selected portions of the plasma membrane of distal tubular cells, was discussed in view of the possibility of paracrine or autocrine effects of GABA on some other uriniferous tubular cells or interstitial cells.

1. Introduction

It is known that gamma-aminobutyric acid (GABA) may play a pivotal role in the renal physiology of natriuresis and hypertension (Hayakawa et al., 2004; Yamakoshi et al., 2007). In support of this physiological information, there have been biochemical studies on the identification in the kidney of glutamic acid decarboxylase (GAD, GABA-synthesizing enzyme) and GABA-receptors as well as GABA itself (Erdö et al., 1991; Párducz et al., 1992; Monasterolo et al., 1996; Sarang et al., 2001, 2008; Takano et al., 2014). With regard to the localization of GABA-signaling molecules, the immunoreactivity for GABA itself has been reported to occur in the cytoplasm of the epithelial cells of the thin and the thick ascending limbs of the loop of Henle, the connecting tubules, and the collecting ducts, but not in glomeruli or vessels of rats (Párducz et al., 1992). On the other hand, the immunoreactivity for GAD has been shown to occur in proximal and distal tubular cells of mice (Liu et al., 1996) and glomeruli and intra-renal arterioles of the cortex in rat kidneys (Takano et al., 2014). The immunoreactivity for $GABA_A$ receptor has been shown to occur mostly in proximal tubules, and that for $GABA_B$ receptor in glomeruli and collecting tubules in the cortex of rat kidneys (Takano et al., 2014), while the binding sites for GABA receptor has been shown to occur in the outer medulla of rats (Beaumont et al., 1984). Since there are some discrepancies in their localization data, further studies remain to be elucidated with a different marker for GABA signaling-related molecule(s) in order to clarify confirmatively sites of synthesis and storage/release of GABA.

In this regard, vesicular inhibitory amino acid transporter (VIAAT) for GABA and glycine has been identified in rodent brain and it localizes the amino acids to synaptic vesicles of central inhibitory neurons (Sagné et al., 1997; Chaudhry et al., 1998). There have recently been studies suggesting that the occurrence of VIAAT is a good immunohistochemical marker of GABAergic signaling in extra-brain tissue cells containing GABA and its synthesizing

* Corresponding author at: Electron Microscopy Unit, Department of Anatomy, Faculty of Medicine, Khon Kaen University, Muang, Khon Kaen 40002, Thailand.
E-mail address: wiphawi@kku.ac.th (W. Hipkaeo).

https://doi.org/10.1016/j.aanat.2018.11.002
0940-9602/

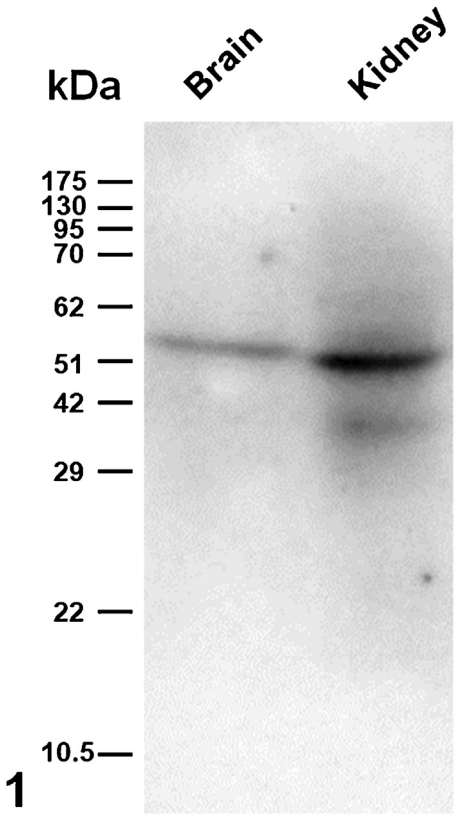

Fig. 1. Immunoblot of kidney and brain of mice for VIAAT. Distinct bands at a size of 55 kDa are clearly recognized in both tissues.

enzyme termed GAD, such as the pancreatic islets and salivary glands (Gammelsaeter et al., 2004).

Considering this information described above, the present study was undertaken to localize VIAAT in the kidney of mice by immunohistochemistry at light and electron microscopic levels.

2. Materials and methods

2.1. Animals

Male ICR mice of postnatal 8 weeks were purchased from National Laboratory Animal Center (NLAC), Bangkok, Thailand and grown under standard laboratory conditions with free access to food and water. All procedures were conducted in accordance with Guidelines for the Care and Use of Laboratory Animals at Khon Kaen University. This study was approved by the Animal Ethics Committee of Faculty of Medicine, Khon Kaen University, based on the Ethics of Animal Experimentation of the National Research Council of Thailand (Reference No. AEMDKKU 003/2017).

2.2. Western blotting analysis

Mice were sacrificed by deep anesthesia with pentobarbital sodium (100 mg/body weight) and subsequently the kidneys were extirpated and immediately put into liquid nitrogen. The frozen mouse kidneys were homogenized in a lysis buffer composed of 20 mM Tris-HCl (pH 8.5), 20 mM KCl, 10 mM EDTA (pH 8.0), 250 mM sucrose, and the proteinase inhibitor cocktail (Roche; Mannheim, Germany). After centrifugation at 3000 rpm, supernatants were examined for the protein concentration using NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific; Wilmington, DE USA). The total proteins of 40 μg from each lysate were individually boiled for 10 min in 2X SDS sample buffer and

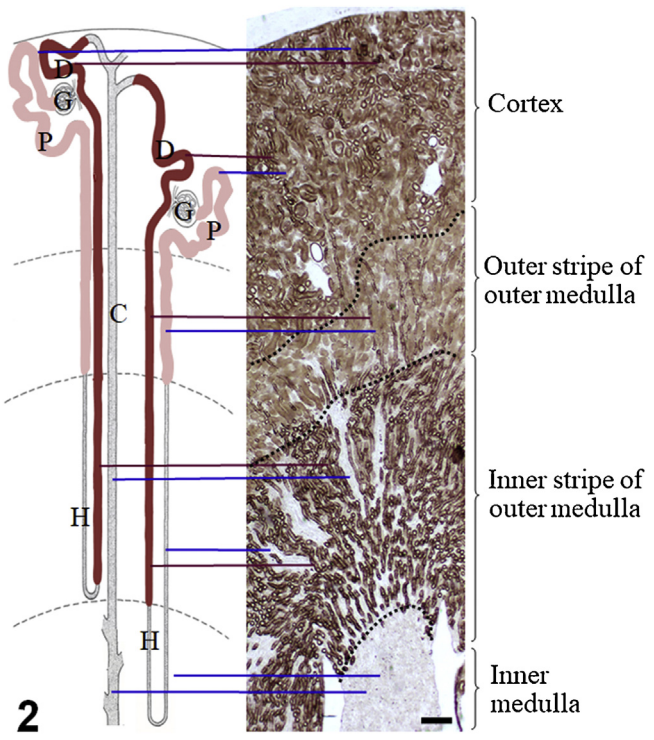

Fig. 2. Immuno-DAB light micrograph of adult mouse kidney in a transverse section (right) in association of a schematic drawing of various segments of nephron in renal cortex and medulla (left). Note appearance of intensely immunopositive tubular profiles in a high population density in the inner stripe of outer medulla, in a lower population density in the outer stripe of outer medulla, and in a moderate density in the cortex, and also note actually no immunopositive tubules in the inner medulla. Intensely immunopositive (presently supposed and later confirmed "distal" (D)) tubules are shown with dense brown-color (representing high immunointensity), while faintly immunostained (presently supposed and later confirmed "proximal" (P)) tubules are shown with light brown color in the drawing. In addition, corresponding portions in micrograph and drawing are indicated by thin solid (brown & blue) lines. C: collecting tubule; G: glomerulus; H: Henle's loop. Bar represents 200 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

subjected to SDS/10% PAGE electrophoresis. They were then electrophoretically transferred to a PVDF membrane (GE Healthcare; Buckinghamshire, UK). After blocking the non-specific binding sites in 5% skim milk (wt/vol)/TBS/0.3%tween-20, the membrane was incubated for overnight at 4 °C with the rabbit IgG against VIAAT (0.1 μg/ml, a kind gift of Prof M Watanabe) in 5% skim milk (wt/vol)/TBS/0.1%tween-20 and then treated with peroxidase-conjugated anti-rabbit IgG (dilution 1: 2500) for 1 h, at RT, and the immunoreactive proteins were visualized using the ECL prime western blotting substrate (GE Healthcare; Buckinghamshire, UK).

2.3. Immunohistochemistry

Mice, under pentobarbital sodium anesthesia, were perfused through the heart with 10 ml physiological saline, followed by 10 ml 4% paraformaldehyde/PBS. The kidneys were removed, then postfixed with the same fixative for overnight. Specimens were dipped into 30% sucrose/PBS for cryoprotection. Cryosections of 20 μm thickness were made on a cryostat and incubated with 0.3% H_2O_2/methanol for 10 min to inhibit intrinsic peroxidase activity, then 10% normal goat serum/PBS for 30 min to prevent non-specific antibody binding. Sections were incubated at room temperature overnight with the rabbit IgG against VIAAT (1 μg/ml) as used in our previous study (Toomsan et al., 2015). The specificity of the antibody was confirmed previously (Kudo et al., 2012). In addition, as a method for identification of the VIAAT-immunopositive

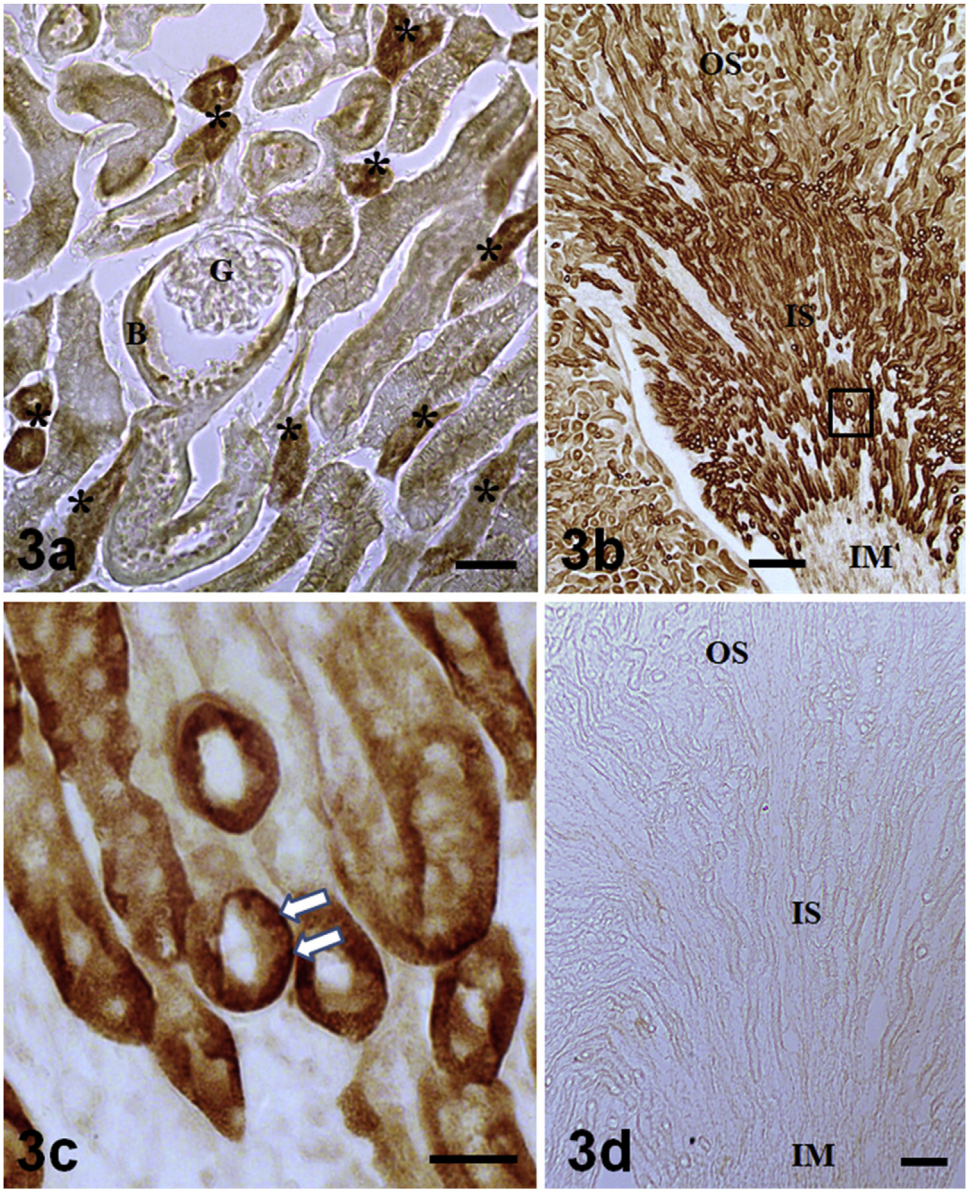

Fig. 3. (a–d) Immuno-DAB light micrograph for VIAAT in the cortex (3a) and inner medulla (3b, 3c), and that of the specimen immunoabsorbed with the synthetic antigen, showing no significant immunoreaction in any portions of section (3d). Note the occurrence of uriniferous tubules showing intense immunoreactivity (*) mixed with those showing faint immunoreactivity, some of which were directly continuous to the Bowman capsule (B) enclosing immunonegative glomerulus (G) (3a). Also note a dominance of intensely immunopositive tubules in the outer medulla composed of outer and inner stripes (OS, IS) with a lack of the immunoreactivity in the inner medulla (IM) (3b). A portion of the inner stripe enclosed by a rectangle in (3b) is shown in (3c). Note more dominant immunoreactivity for VIAAT in the basal portion of intensely immunopositive epithelial cells (arrows in 3c). Bars represent 75 μm (3a), 200 μm (3b, 3d), 25 μm (3c).

tubules, rabbit IgG against Aquaporin 1 (AQP1) as a proximal tubule marker (1 ug/ml, Merck, Darmstadt, Germany, Georgas et al., 2008) and calbindin/spot35 as a collecting tubule marker (Georgas et al., 2008, 1 ug/ml; for the immune-specificity, refer to Yamakuni et al. (1984) and Yamamoto et al. (1991)) were used together with the VIAAT antibody in the alternate immunostaining method using several sets of two consecutive mirror sections. The sections were incubated for 1 h at room temperature with biotinylated goat anti-rabbit IgG (H + L) (Abcam; Cambridge, MA USA). For visualization of the antigen-antibody reaction sites with diaminobenzidine (DAB) reaction as the marker, the sections were then treated by ABC kit (Vector Laboratories, Burlingame, CA USA).

In immuno-DAB electron microscopy, some of the sections were postfixed with 0.5% OsO_4 in 0.1 M phosphate buffer and embedded in Epon after *en-bloc* staining with 0.1% uranyl acetate. Ultrathin sections were observed under JEM1010 transmission electron microscope (Jeol; Tokyo, Japan).

For pre-embedding immuno-gold electron microscopy, cryostat-sections, after incubation with the antibody (5 μg/ml) overnight, were reacted with goat anti-rabbit IgG covalently linked with ultrasmall gold particles (1:100 in dilution; Aurion, Hatfield, PA, USA). Following silver enhancement using an Aurion R-GENT SE-LM silver enhancement kit (Aurion, Hatfield, PA, USA), the sections were osmicated, dehydrated, and directly embedded in Epon. Ultrathin sections were prepared and stained with both uranyl acetate and lead citrate for observation.

In the antigen-absorption test for control of the immunohistochemistry, the antibody was pre-absorbed with synthetic VIAAT antigen (100 μg/ml) and sections were incubated with the absorption solution for 1 h at room temperature and subsequently treated in the same procedure as the regular immunoreaction described

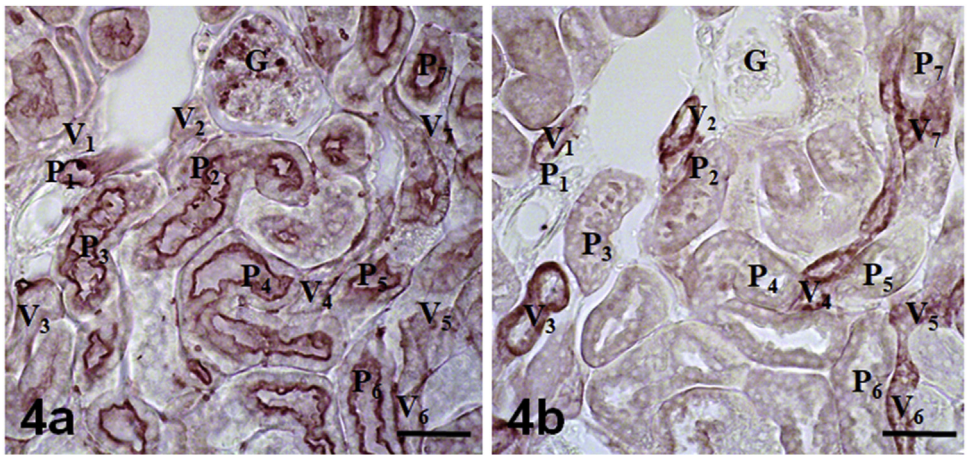

Fig. 4. (a, b) Immuno-DAB light micrographs of two adjacent mirror sections immunostained for AQP1 as a proximal tubule marker (4a) and for VIAAT (4b). Note that VIAAT-immunopositive tubules (V_1–V_7) are immunonegative for AQP1 (P_1–P_7). G: glomerulus. Bars represent 50 μm.

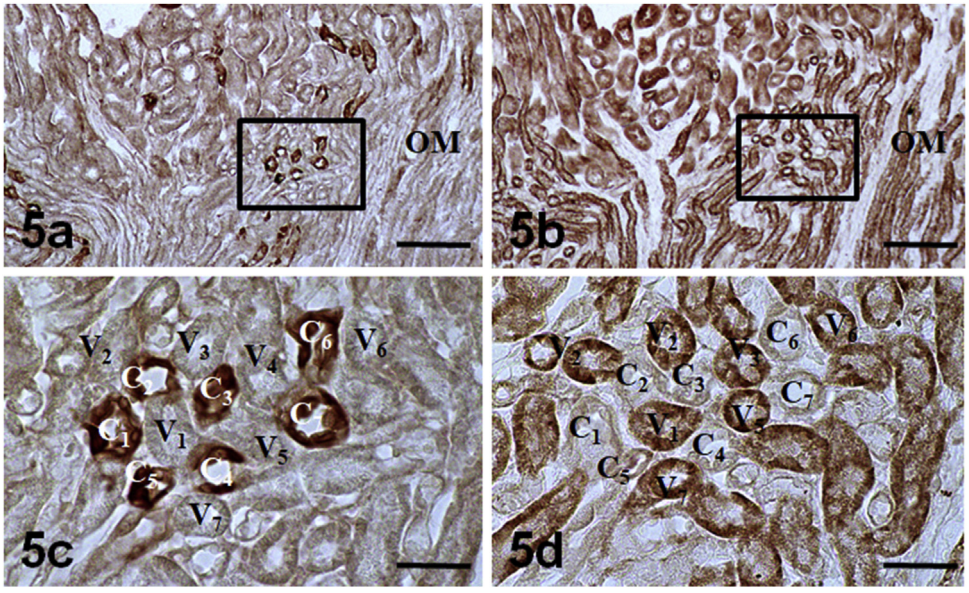

Fig. 5. (a–d) Immuno-DAB light micrographs of two adjacent mirror sections immunostained for calbindin/spot 35 as a collecting tubule marker (5a,c) and for VIAAT (5b,d). Regions enclosed by rectangles in 5a and b are shown at higher magnification in 5c and d, respectively. Note that VIAAT-immunopositive tubules (V_1–V_7) are immunonegative for calbindin/spot35 (C_1–C_7). By this way of elimination, intensely VIAAT-immunopositive tubules were identified as distal tubules. Bars represent 100 μm (5a, 5b), 50 μm (5c, 5d).

above. In addition, the omission of the primary antibody was done for another control in immunohistochemistry.

3. Results

In Western blotting, a single distinct band for VIAAT was detected in homogenates of the kidney of adult mice, while a stronger band at the same size was also detected in homogenates of the total brain of adult mice. The size was in accord with the authentic molecular size for VIAAT in the brain as already reported with the same antibodies as used in this study (Kudo et al., 2012), (Fig. 1).

In immuno-light microscopy, although the immunoreactivity for VIAAT was seen more or less positive in almost all thick uriniferous tubules in the cortex, it was much more intense in tubules characterized by relatively smooth luminal contours. The glomerulus was faintly immunoreactive or actually immunonegative. In the outer medulla, intense immunoreactivity for VIAAT was seen in thick tubules with relatively smooth luminal contours, while other thicker and thinner tubules without significant immunoreactivity were interposed among the former intensely immunopositive tubules. The intensely immunopositive tubules were dominant in number, especially in the inner stripe of outer medulla. In contrast, no immunoreaction was actually discerned in any tubules in the inner medulla. In the intensely immunoreactive tubules, the immunoreactivity was dominantly localized in the basal and infranuclear portions of the epithelial cells, while their nuclei were free of the immunoreaction. No distinct immunoreactivity was seen in capillary endothelia or interstitial fibroblasts throughout the kidney (Figs. 2, 3 a–c).

When several sets of two consecutive mirror sections of the kidney were immunostained for VIAAT and AQP1 or calbindin/spot35, VIAAT-immunopositive tubules were immunonegative for either of the two proteins in both the outer medulla and the cortex (Figs. 4 and 5).

In control experiments by both antigen-absorption test and omission of the primary antibody, no significant immunoreaction was detected in any portions of the specimens (Fig. 3d).

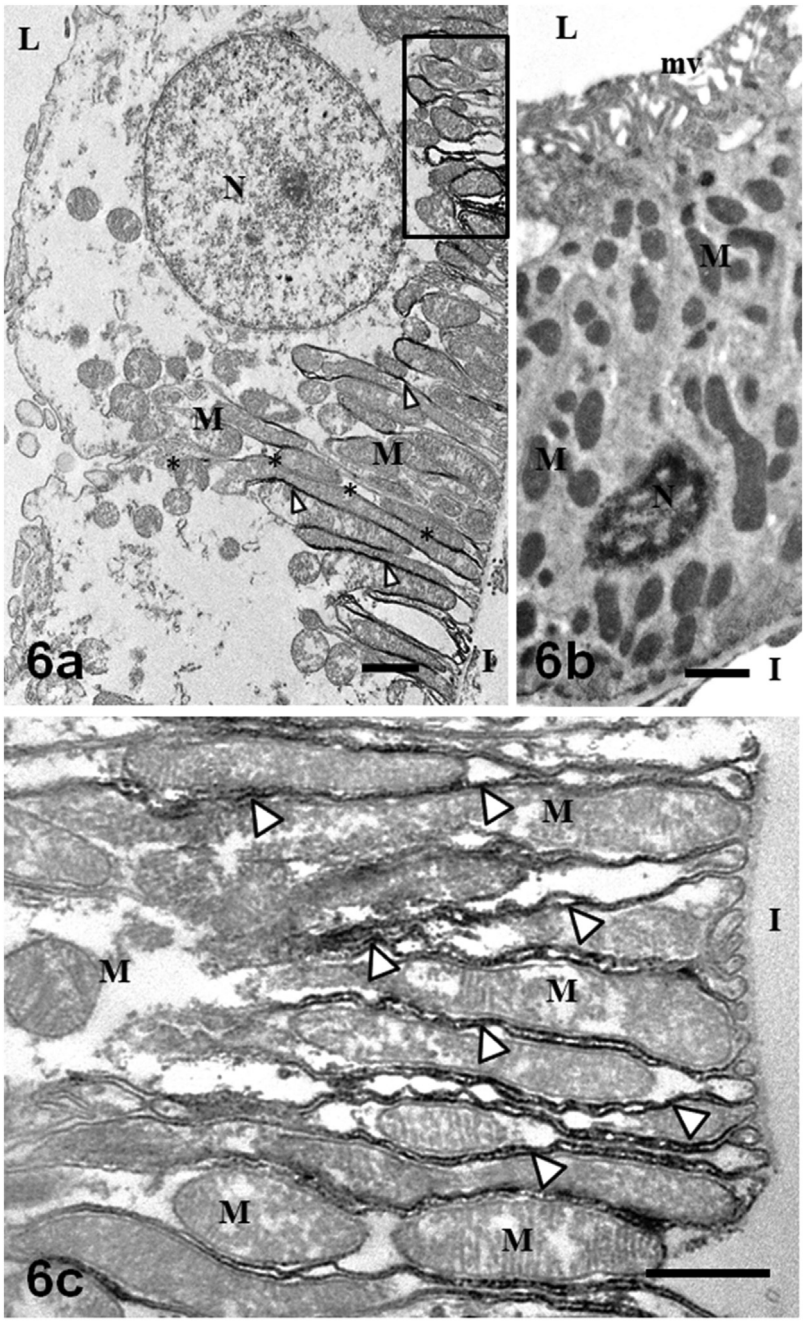

Fig. 6. (a–c) Immuno-DAB electron micrographs of distal (6a) and proximal (6b) tubular epithelial cells, and a basal cell portion of distal tubular cell corresponding to that enclosed by a rectangle in 6a (6C). Note the immunoreactivity along the plasma membranes in close association with mitochondria inserted in the basal infoldings (white arrow heads) and lateral plasma membrane (asterisks). I: interstitial space, L: tubular lumen, M: mitochondria, mv: microvilli, N: nucleus. Bars represent 1 μm (6a, 6b), and 800 nm (6c).

In immuno-DAB electron microscopy of the intensely immunopositive tubules, electron-dense immunoreaction materials were deposited in the infranuclear portions rich in mitochondria of the epithelial cells characterized by well-developed basal infoldings, while no significant immunoreaction deposits were seen in the apical/supranuclear portions of the cells (Fig. 6a). In contrast, no significant immunoreactive materials was discerned in entire portions of tubular epithelia equipped with abundant microvilli in their luminal portions (Fig. 6b). At higher magnification, the immunoreaction materials were associated with the basal infoldings in parallel to the long axis of elongated mitochondria, and also with the lateral plasma membranes (Fig. 6a, c).

In immuno-gold electron microscopy, gold particles representing the immunoreaction were localized on the basal portions of the lateral plasma membranes and the membranes of the basal infoldings. No gold particles were seen in association with any intracellular vesicles and vacuoles, or the basal and apical plasma membranes and their subjacent cytoplasm (Fig. 7a,b).

4. Discussion

Since a distinct band with the size corresponding to the authentic brain VIAAT of mouse (Sagné et al., 1997) was detected in the kidney as well as the brain in the present immunoblotting, and because no significant immunoreaction was discerned in any por-

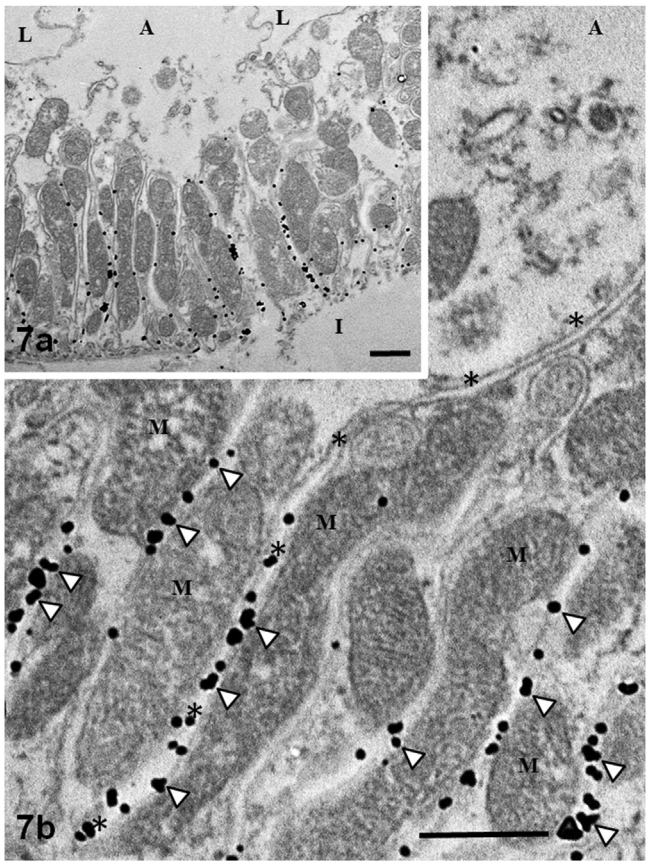

Fig. 7. (a,b) Immuno-gold electron micrographs of distal tubular cell at lower (7a) and higher (7b) magnifications. Note localization of gold particles for VIAAT on the plasma membranes of basal infoldings (white arrow heads) and of basal portions of lateral plasma membranes (asterisks). A: apical cytoplasm. I: interstitial space. L: tubular lumen. M: mitochondria. Bars represent 1 μm.

tions of the kidney in immunohistochemistry for controls by the antigen-absorption test and omission of the primary antibody, it is reasonable to interpret that the present immunohistochemical positivities in the kidney are due to the authentic VIAAT, although the final confirmation has to be clarified by examination of the kidney from gene-knockout mice for VIAAT.

Since all VIAAT-immunopositive tubules were revealed to be immuno-negative for either AQP1, a proximal tubule marker, or calbindin/spot35, a collecting tubule marker, in the analysis by the alternate immunostaining with the two antibodies using several sets of two consecutive mirror sections, it is highly possible to conclude by this way of elimination that a majority, if not all, of VIAAT-immunopositive tubules are the distal uriniferous tubules. Needless to mention, further analyses by the double immunostaining on one and the same sections using an antibody for a distal tubule-specific marker generated in animals other than rabbits remain to be done for further confirmation of this conclusion because of the antibody for VIAAT generated in rabbits. However, such non-rabbit-originated antibodies specific for the distal tubule are not available at hand around us, unfortunately. The identification of most, if not all, immunopositive tubules as the distal tubules by this method of elimination is further supported by a feature of well-developed basal infoldings in the VIAAT-immunopositive cells. Therefore, the present finding strongly suggests that the intrarenal GABA-signaling, whose occurrence has been reported by others (Monasterolo et al., 1996; Hayakawa et al., 2004; Yamakoshi et al., 2007), is intensively exerted in the epithelial cells of the distal tubules, especially more distinctly in the outer medulla. This finding is well compatible with that of GABA localization in the kidney of rats reported by Párducz et al. (1992). The reason why the GABA-signaling is rather dominantly exerted in the outer medullary portions of the distal tubules remains to be further elucidated.

On the other hand, the present ultrastructural localization of VIAAT-immuno- reactivity on the plasma membrane of the basal infoldings and of basal portions of the lateral plasma membranes in the distal tubule cells is enigma. It is because VIAAT was originally identified as a protein in synaptic vesicle membranes in the brain (Chaudhry et al., 1998). Its localization in the synaptic vesicles has already been confirmed in the brain by using the same antibody as employed in the present study (Kudo et al., 2012). Therefore, VIAAT-immunoreactivity had been expected to be localized in some intracellular membranes including vesicles and vacuoles of the uriniferous epithelial cells, but the result was not the case. In a previous study of VIAAT-localization in the pancreatic islets as its first target in extra-brain tissue cells (Hayashi et al., 2003; Gammelsaeter et al., 2004), its immunoreactivity was shown to be localized in the membrane of the endocrine granules of islet α cells as expected from its localization in synaptic vesicles in the brain. Some vesicular membrane proteins can incorporate into the plasma membrane when the two membranes are fused to each other at sites of exocytosis of the vesicles. Such a feature has been represented by the occurrence of immunoreactivity for choline transporter on the plasma membrane of presynaptic nerve endings, although a majority of the immunoreactivity was shown to occur on intracellular membranes of synaptic vesicles (Ferguson et al., 2003). A more suggestive/supportive fact is the localization of inositol triphosphate (IP_3) receptor. Although IP_3 receptor is generally considered to be localized in membranes of the intracellular Ca^{2+} reservoir, that is, endoplasmic reticulum, there have been data showing that its subtype, especially IP_3 receptor 2 may be localized in the plasma membrane (Vermassen et al., 2004; Dingsdale et al., 2012). On the other hand, there have been studies showing that several isoforms of a channel protein family are localized to intracellular vesicles, and also can function in the plasma membranes (Bezzerides et al., 2004; Oancea et al., 2006; Brauchi et al., 2008).

Considering these data so far published, several possible interpretations for the present enigmatic VIAAT-localization in ultrastructure are possible: the one is that the present VIAAT-immunoreactivity solely located on the plasma membranes may represent an extreme case of incorporation of VIAAT, which would be originally localized in some vesicles, into the selected domains of plasma membranes as the site of exocytosis under some yet unknown conditions. Another is that a yet-identified plasma membranous GABA transporter (GAT) cross-reacts with the present VIAAT antibody. This is, however, less likely because any of the total four already known GAT isoforms do not show any homology in the molecular structure to VIAAT (Zhou and Danbolt, 2013). Regarding the localization of known GAT, a previous study has shown that GAT2 is localized in the basal membrane of tubular cells in the cortex of rat kidney without clarification about whether proximal or distal tubules were involved (Takano et al., 2014). Although no clear interpretation is possible for this enigmatic localization at present, if the first possible interpretation is the case, GABA would be released into the intrarenal interstitial space through the basolateral plasma membranes, resulting in its paracrine or autocrine effects on some cells comprising the nephron or even on interstitial cells. These effects may be possible in mouse kidneys, considering the occurrence of immunoreactivity for $GABA_A$ receptor in proximal tubules, and that for $GABA_B$ receptor in glomeruli and collecting tubules in rat kidneys (Takano et al., 2014), and the binding sites for GABA receptor in the outer medulla of rat kidneys (Beaumont et al., 1984).

Admittedly, further studies are necessary to resolve such a discrepancy in the localization of VIAAT between the plasma

membranes in the uriniferous tubular epithelium and the intracellular/vesicular membranes in neuronal and neuroendocrine cells. Regardless of this issue, the present immune-light and electron microscopic localization of VIAAT sheds more light than before on our understanding of the GABA signaling system that might contribute to modulation of the renal function including the urinary electrolyte balance.

Acknowledgements

The authors sincerely thanks Mr D Hipkaeo for his technical assistance. This work was supported by the Faculty of Medicine, Khon Kaen University, Thailand through WS&WH [grant number I57318].

References

Beaumont, K., Healy, D.P., Fanestil, D.D., 1984. Autoradiographic localization of benzodiazepine receptors in the rat kidney. Am. J. Physiol. 247, F718–F724.

Bezzerides, V.J., Ramsey, I.S., Kotecha, S., Greka, A., Clapham, D.E., 2004. Rapid vesicular translocation and insertion of TRP channels. Nat. Cell Biol. 6, 709–720.

Brauchi, S., Krapivinsky, G., Krapivinsky, L., Clapham, D.E., 2008. TRPM7 facilitates cholinergic vesicle fusion with the plasma membrane. Proc. Natl. Acad. Sci. U. S. A. 105, 8304–8308.

Chaudhry, F.A., Reimer, R.J., Bellocchio, E.E., Danbolt, N.C., Osen, K.K., Edwards, R.H., Storm-Mathisen, J., 1998. The vesicular GABA transporter, VGAT, localizes to synaptic vesicles in sets of glycinergic as well as GABAergic neurons. J. Neurosci. 18, 9733–9750.

Dingsdale, H., Voronina, S., Haynes, L., Tepikin, A., Lur, G., 2012. Cellular geography of IP_3 receptors, STIM and Orai: a lesson from secretory epithelial cells. Biochem. Soc. Trans. 40, 108–111.

Erdö, S.L., Dobó, E., Párducz, A., Wolff, J.R., 1991. Releasable GABA in tubular epithelium of rat kidney. Experientia 47, 227–229.

Ferguson, S.M., Savchenko, V., Apparsundaram, S., Zwick, M., Wright, J., Heilman, C.J., Yi, H., Levey, A.I., Blakely, R.D., 2003. Vesicular localization and activity-dependent trafficking of presynaptic choline transporters. J. Neurosci. 23, 9697–9709.

Gammelsaeter, R., Frøyland, M., Aragón, C., Danbolt, N.C., Fortin, D., Storm-Mathisen, J., Davanger, S., Gundersen, V., 2004. Glycine, GABA and their transporters in pancreatic islets of Langerhans: evidence for a paracrine transmitter interplay. J. Cell Sci. 117, 3749–3758.

Georgas, K., Rumballe, B., Wilkinson, L., Chiu, H.S., Lesieur, E., Gilbert, T., Little, M.H., 2008. Use of dual section mRNA in situ hybridisation/immunohistochemistry to clarify gene expression patterns during the early stages of nephron development in the embryo and in the mature nephron of the adult mouse kidney. Histochem. Cell Biol. 130, 927–942.

Hayakawa, K., Kimura, M., Kasaha, K., Matsumoto, K., Sansawa, H., Yamori, Y., 2004. Effect of a gamma-aminobutyric acid-enriched dairy product on the blood pressure of spontaneously hypertensive and normotensive Wistar–Kyoto rats. Br. J. Nutr. 92, 411–417.

Hayashi, M., Otsuka, M., Morimoto, R., Muroyama, A., Uehara, S., Yamamoto, A., Moriyama, Y., 2003. Vesicular inhibitory amino acid transporter is present in glucagon-containing secretory granules in alphaTC6 cells, mouse clonal alpha-cells, and alpha-cells of islets of Langerhans. Diabetes 52, 2066–2074.

Kudo, T., Uchigashima, M., Miyazaki, T., Konno, K., Yamasaki, M., Yanagawa, Y., Minami, M., Watanabe, M., 2012. Three types of neurochemical projection from the bed nucleus of the stria terminalis to the ventral tegmental area in adult mice. J. Neurosci. 32, 18035–18046.

Liu, Z.H., Striker, L.J., Hattori, M., Yang, C.W., Striker, G.E., 1996. Localization of glutamic acid decarboxylase in the kidneys of nonobese diabetic mice. Nephron 72, 662–666.

Monasterolo, L.A., Trumper, L., Elías, M.M., 1996. Effects of gamma-aminobutyric acid agonists on the isolated perfused rat kidney. J. Pharmacol. Exp. Ther. 279, 602–607.

Oancea, E., Wolfe, J.T., Clapham, D.E., 2006. Functional TRPM7 channels accumulate at the plasma membrane in response to fluid flow. Circ. Res. 98, 245–253.

Párducz, A., Dobó, E., Wolff, J.R., Petrusz, P., Erdö, S.L., 1992. GABA-immunoreactive structures in rat kidney. J. Histochem. Cytochem. 40, 675–680.

Sagné, C., El Mestikawy, S., Isambert, M.F., Hamon, M., Henry, J.P., Giros, B., Gasnier, B., 1997. Cloning of a functional vesicular GABA and glycine transporter by screening of genome databases. FEBS Lett. 417, 177–183.

Sarang, S.S., Lukyanova, S.M., Brown, D.D., Cummings, B.S., Gullans, S.R., Schnellmann, R.G., 2008. Identification, coassembly, and activity of gamma-aminobutyric acid receptor subunits in renal proximal tubular cells. J. Pharmacol. Exp. Ther. 324, 376–382.

Sarang, S.S., Plotkin, M.D., Gullans, S.R., Cummings, B.S., Grant, D.F., Schnellmann, R.G., 2001. Identification of the gamma-aminobutyric acid receptor beta(2) and beta(3) subunits in rat, rabbit, and human kidneys. J. Am. Soc. Nephrol. 12, 1107–1113.

Takano, K., Yatabe, M.S., Abe, A., Suzuki, Y., Sanada, H., Watanabe, T., Kimura, J., Yatabe, J., 2014. Characteristic expressions of GABA receptors and GABA producing/transporting molecules in rat kidney. PloS One 9, e105835.

Toomsan, Y., Chomphoo, S., Pakkarato, S., Watanabe, M., Kondo, H., Hipkaeo, W., 2015. Localization of vesicular inhibitory amino acid transporter (VIAAT) in the submandibular salivary gland throughout the postnatal development of mice. Int. J. Morphol. 33, 113–118.

Vermassen, E., Parys, J.B., Mauger, J.P., 2004. Subcellular distribution of the inositol 1,4,5-trisphosphate receptors: functional relevance and molecular determinants. Biol. Cell 96, 3–17.

Yamakoshi, J., Fukuda, S., Satoh, T., Tsuji, R., Saito, M., Obata, A., Matsuyama, A., Kikuchi, M., Kawasaki, T., 2007. Antihypertensive and natriuretic effects of less-sodium soy sauce containing gamma-aminobutyric acid in spontaneously hypertensive rats. Biosci. Biotechnol. Biochem. 71, 165–173.

Zhou, Y., Danbolt, N.C., 2013. GABA and glutamate transporters in brain. Front. Endocrinol. (Lausanne) 4, 165.

Yamakuni, T., Usui, H., Iwanaga, T., Kondo, H., Odani, S., Takahashi, Y., 1984. Isolation and immunohistochemical localization of a cerebellar protein. Neurosci. Lett. 45, 235–240.

Yamamoto, M., Yamakuni, T., Takahashi, Y., Kondo, H., 1991. Transient appearance of immunoreactivity for Ca-binding protein (spot 35-calbindin) in small principal neurons in the superior cervical ganglion of pre-weanling rats. J. Auton. Nerv. Syst. 35, 25–31.