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VNUT PLAYS AN IMPORTANT ROLE IN VESICULAR STORAGE AND SUBSEQUENT EXOCYTOSIS OF ATP FROM BLADDER EPITHELIUM UPON MECHANICAL STRETCH STIMULATION

Hypothesis / aims of study

Several substances including ATP, ACh, NO, PG and NGF, are released from urothelial cells following physical and chemical stimulation, among which ATP plays a central role for afferent nerve signal transduction mechanism (1). However, molecular mechanisms and pathways of ATP release are largely unknown. ATP has been reported to be released at least 6 different pathways/mechanisms (2). Here we have investigated exocytotic release of ATP from urothelium, since exocytosis is a more adjustable and positive mechanism for secretion. We confirmed VNUT (3) localized in urothelial cell of mouse bladder. VNUT plays an essential role in vesicular storage of ATP in the ATP-secreting cells. We have investigated the accumulated ATP in secretory vesicle via VNUT and exocytotic release of ATP from urothelium upon stretch stimulation in urothelial cells.

Study design, materials and methods

WT (C57BL/6Cr) mice were used. The experiments were performed by mouse primary urothelial cell culture from WT and VNUT RNA interference. VNUT siRNA was transfected to mouse primary urothelial cell culture. RT-PCR: Extracted *m*-RNA from mouse urothelial primary culture cells and confirmed the expression of VNUT gene. Immunostaining: Expression of VNUT protein was confirmed using an anti-VNUT antibody in mouse bladder specimen and urothelial primary culture cells. Quinacrine-labeling: Used to locate intracellular stores of ATP bound to peptides, displaying a granular fluorescence. VNUT-RFP: (VNUT was labeling by red fluorescent protein) was transfected to mouse primary urothelial cell, and merged with Fluorescent-ATP/Quinacrine. The visualized Exocytosis: Quinacrine-labeling vesicle upon stimulation with a pipet in urothelial cell. Photon imaging of ATP release: ATP release from the primary urothelial cultures upon mechanical stretch stimulation was investigated.

Results

VNUT gene and protein was highly expressed in the epithelial layer of mouse bladder tissue (fig.1) and primary urothelial cell culture. Quinacrine positive vesicles are identified in urothelial cell. Co - location between VNUT-RFP and Fluorescent ATP accumulated in vesicle form is demonstrated (fig.3). VNUT-RFP locates in a Quinacrine-labeling vesicle similarly. The dynamics of Quinacrine-labeling vesicle upon a mechanical stimulation in urothelial cell was visualized. Knockdown of VNUT in urothelial primary culture cells markedly reduced ATP release upon mechanical stretch stimulation (fig.3).

Interpretation of results

These findings suggest that VNUT and secretory vesicle containing ATP exist in mouse urothelial cell and urothelium should transmit ATP to afferent signal transduction by the exocytosis pathway. We claim a molecular mechanism(s) underlying ATP release from urothelial cell upon stretch. The stretch stimulation activates the mechanosensor channel such as TRPV4 channel. Subsequent intracellular Ca^{2+} influx might trigger ATP, which is stored to vesicle via VNUT, release from the mechanism of exocytosis.

Concluding message

VNUT dependent vesicular exocytosis was involved in ATP release pathway. Vesicular exocytosis of ATP appears to play an important role in distension of the mouse bladder epithelial cell.

Fig.1. Immunostaining in mouse bladder tissue.(green:VNUT)(Scalebar:50μm)

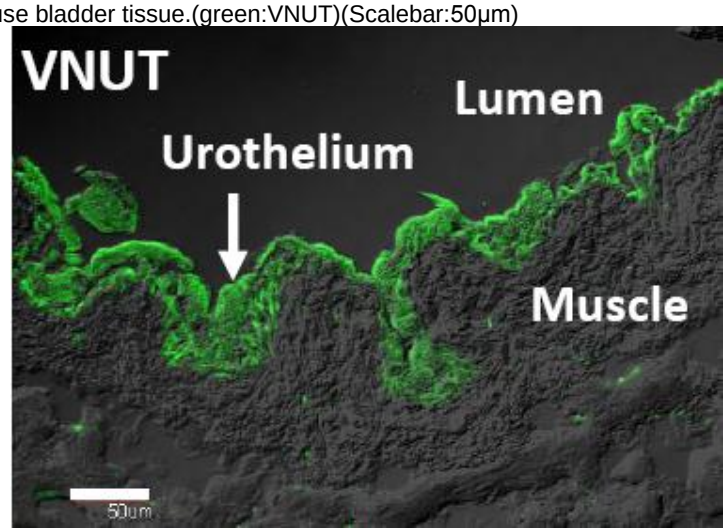


Fig.2. VNUT-RFP and Fluorescent ATP in mouse primary urothelial cell (Scale bar:10μm)

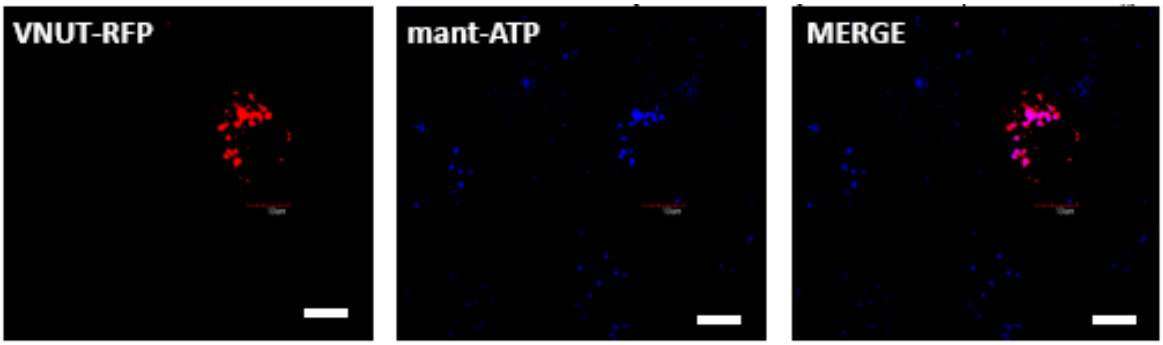
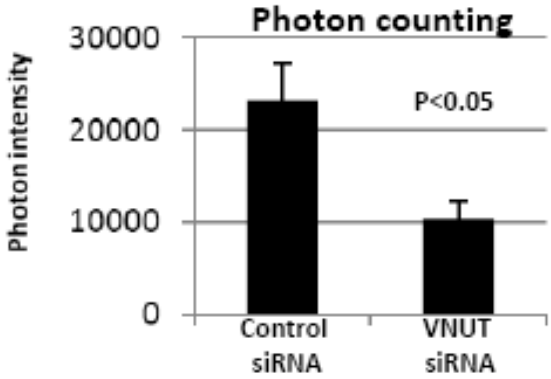


Fig.3. Photon imaging of ATP release (Error bar: mean±S.E.M.) (student's t test)



References

1. Birder LA, et al; Mechanisms of disease: involvement of the urothelium in bladder dysfunction. Nat Clin Pract Urol. (2007) ;4(1):46-54
2. Sabirov RZ, et al; ATP release via anion chann.Purinergic Signal. (2005);1(4):311-28.
3. Sawada K, et al; Identification of a vesicular nucleotide transporter. PNAS. (2008) 15;105(15):5683-6.

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What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**the University of Yamanashi Animal Care and Use Committee**