Review of: "The V-type H+-ATPase is targeted in anti-diuretic hormone control of the Malpighian 'renal' tubules in Aedes aegypti"

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Review: The V-type H^+-ATPase is targeted in anti-diuretic hormone control of the Malpighian 'renal' tubules in*Aedes aegypti,* by Farwa Sajadi and Jean-Paul V. Paluzzi, Department of Biology, York University, Canada.

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V-type H^+-ATPase plays important roles in channel and transporter membrane recycling, lysosomal enzyme activation, and proton secretion in the renal tubules of mammalian. Interestingly, V-type H^+-ATPase plays a postprandial diuretic role in the Malpighian renal tubules of yellow fever mosquito, *Aedes aegypti*. The authors revealed that stimulation with diuretic hormone (DH31) increased assembly of V1 subunit with V0 subunit of V-type H^+-ATPase in the membrane fraction and increased V-type H^+-ATPase activity measured by fluid secretion rate and pH changes in the mosquito Malpighian tubules (MTs). Anti-diuretic neuropeptide CAPA inhibited V-type H^+-ATPase activity by dissociation of the ATP-binding and hydrolysis V1 subunit form the membrane V0 subunit of proton channel via NOS/NO/cGMP/PKG pathway. However, analysis of NO produced by NO synthase and NO action on the V-ATPase is missing.

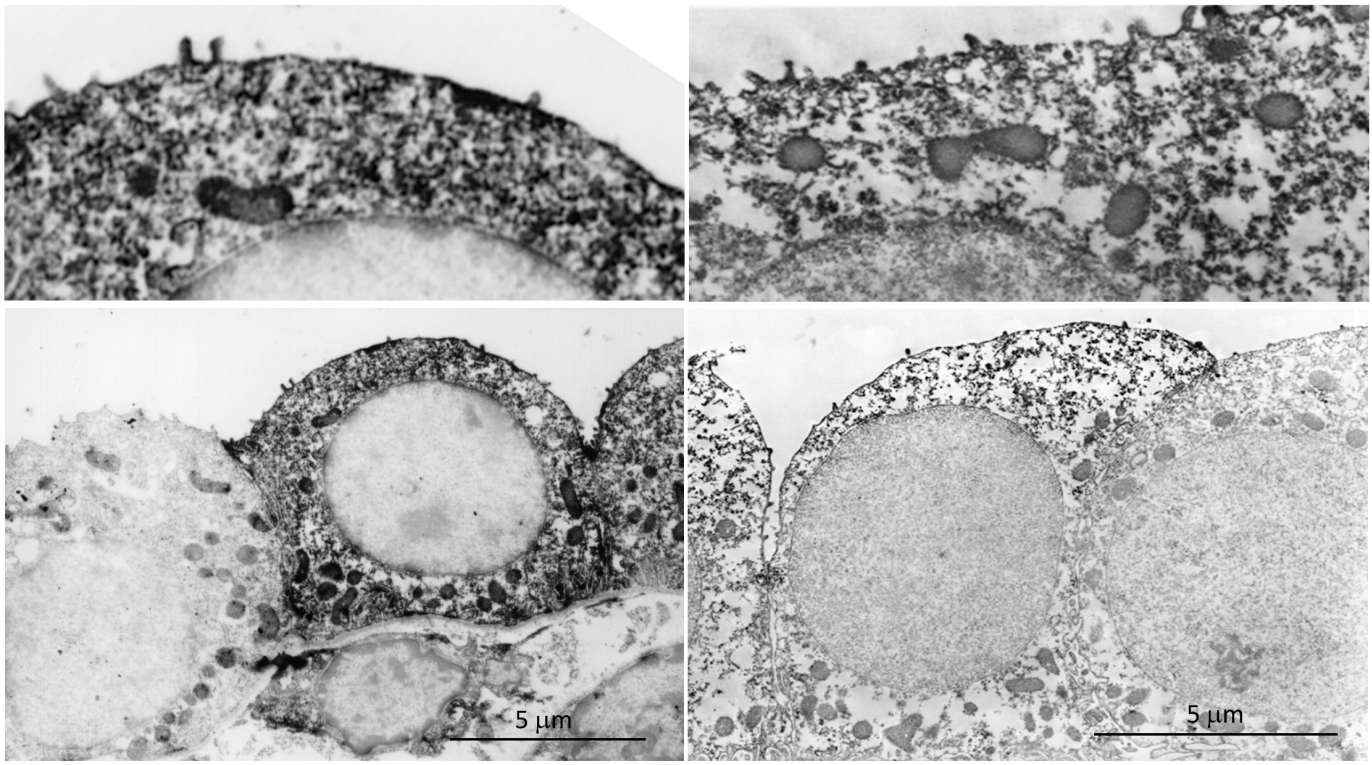
1. In the rat kidney, 3 isoforms of nitric oxide synthase (NOS) expressed; neuronal NOS in the macula densa and Bowman's epithelial cells (J Am Soc Nephrol 1994; 4: 1438-1447), endothelial NOS in the renal vascular endothelial cells, and inducible NOS in the intercalated cells of cortical collecting duct (Am J Physiol 1994; 267: F509-F515), proximal tubules and vascular smooth muscle cells (Kidney Int 1997; 52:1593-1601). Which isoform of NOS is expressed in the insect MTs?

It is interesting to show the NO production by molecular probe DAF-2DA fluorescence (Kidney Int 2005; 67:1890-1898) or measure NO by NO microelectrode probe.

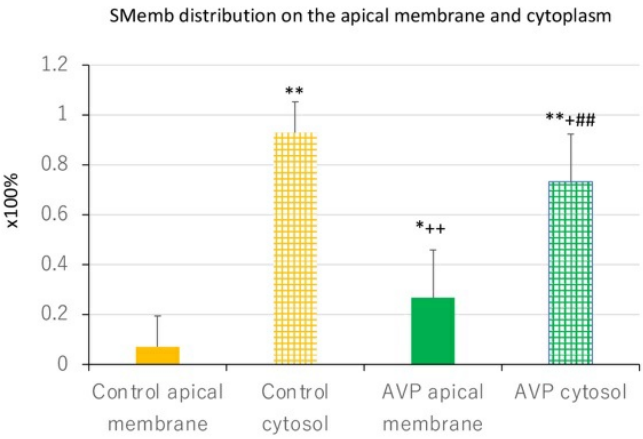
1. Does the exogenous NO donor, sodium nitroprusside (SIN-1) inhibit DH31-induced V1 membrane trafficking and V-ATPase activity? Does LPS/IFN-gamma induce endogenous inducible NOS and NO production in MTs and inhibit V-ATPase activity? Does L-nitroarginine inhibit NOS and reverse V-ATPase activity? Proving the role of NOS/NO/eGMP pathway in V-ATPase activity requires both exogenous and endogenous NO stimulation and second messenger cGMP action (Am J Physiol 1994; 267: F509-F515).
2. The insect antidiuretic neuropeptide CAPA is evolutionarily associated with the vertebrate neuromedin U peptides, first

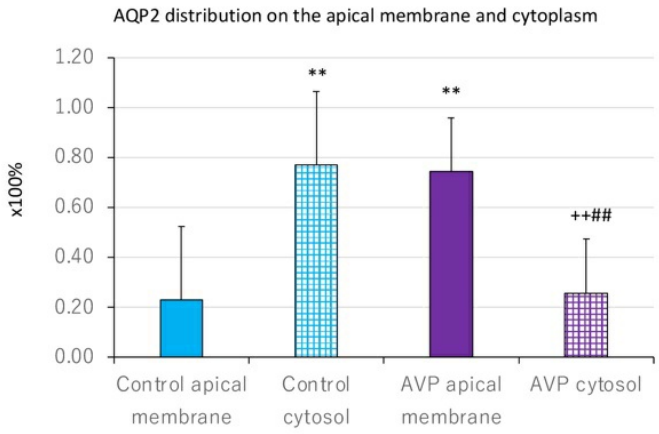
isolated from porcine spinal cord and had potent contractile effect on rat uterus. Physiologically, antidiuretic hormone (ADH) regulates the water channel aquaporin (AQP2) by membrane recycling in the principal cells in the collecting ducts in the kidney. In the absence of ADH, AQP2 is located in the apical vesicles, and under ADH stimulation, AQP2 is inserted into the apical membrane, reabsorbing water exhibiting antidiuretic effects. V-ATPase plays an important role in the membrane recycling of AQP2 in the kidney. Do mosquitoes have water channels like AQP2? It is reasonable to explain the antidiuretic effect of CAPA by regulating the membrane recycling of water channels via V-ATPase.

3. It is also interesting to investigate the role of cytoskeletons in the membrane recycling of V1 subunit from cytosol to apical membrane of MTs. Our preliminary data of embryonic smooth muscle myosin (SMemb) aggregated around apical vesicles with V-ATPase by ADH(AVP) stimulation in the rat collecting duct (Tojo A, Kimura K, Nagai R, Omata M. Non-muscle myosin heavy chain in rat cortical collecting duct. J Am Soc Nephrol 1995; 6 (3):330).



Control AVP (0.15 mU/min for 20min)





SMemb expressed in the principal cells of collecting duct, and SMemb aggregated around cytoplasmic vesicles after AVP stimulation. Immunogold labeling for AQP2 mainly located in the cytoplasm in the control, whereas AQP2 translocated to apical membrane after AVP stimulation. *p<0.05, **p<0.01 vs. Control apical membrane, +p<0.05, ++p<0.01 vs. Control cytosol, ##p<0.o1 vs. AVP apical membrane. (unpublished data from poster of ASN 1995).

1. In the schema of Figure 8, most readers are not familiar with mosquitoes, so it is better to show the microanatomy of the Malpighian ‘renal’ tubules of *Aedes aegypti*.

Na^+-K^+-ATPase (NKA) is the major pump of most of the cells in the basolateral membrane, so NKA in the apical membrane seems strange. The Na^+/H^+ exchanger (NHE). located in the apical membrane of renal proximal tubule, reabsorbs Na^+, excretes H^+ into the lumen, reacts with HCO_3^- to H_2CO_3, and produces H_2O and CO_2 by carbonic anhydrase (CA). In the MTs, expression of NHE could locate in the apical membrane and excrete Na^+ with exchange H^+. Water may be generated with H^+ and HCO_3^- by CA. AQP isoforms also could exist in the apical vesicles in the MTs, and could show the membrane recycling of AQP.

Minor comments

1. Statistical difference markers in Figure 1-4, Figure 7, and Fig 1S as shown a, b, ab, *, ** should be explained with precise comparison forms such as “p<0.05 vs. control”.
2. In the legend of Figure 7, corrected cell fluorescence (CTCF) should be (CCF).