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The Role of the Voltage-gated Proton Channel Hv1 in Vesicular Transportation

［１］Organization

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［２］Purpose of research (size: 10.5 point)

Vesicular transport is a major cellular activity, responsible for molecular traffic by a variety of specific membrane-enclosed compartments. The selectivity of transport is a key to maintaining the functional organization of the cell. Vesicular traffic itself exquisitely depends on pH, which emphasizes the crucial importance of pH regulation in the secretory pathway.

The voltage-gated proton channel Hv1 is a potent acid extruder that participates in the extrusion of the intracellular acid. In this project, our purpose is to elucidate the role of Hv1 in vesicular transportation such as how Hv1 raglulates the exocytosis. In detailes, at first, we determined the subcellular localization of Hv1 in secretory cells. Second, we investigated how Hv1 affects the secretory function in secretory cells such as insulin secretion. Furthermore, in order to elucidate the new biological function of Hv1 in vesicular transport, we also investigated the gating characteristics of Hv1.

［３］ Outcome (size: 10.5 point)

（３－１）Results of the research

(1) Hv1 is expressed in the secretion granules of secretion cells

A strong signal of Hv1 was observed in almost all the cells in the human and mouse islets of Langerhans, whereas the Hv1 signal in the exocrine pancreas was very weak (Fig. 1A, a and c). Immunostaining of serial sections of the human (Fig. 1A, a and b) and mouse (Fig. 1A, c and d) pancreases, and the mouse ß-cell line ßTC-6 (Fig. 1A, e and f) for Hv1 and insulin showed that Hv1 colocalizes with insulin in pancreatic islets.

Immunofluorescence staining of the mouse pancreatic cryosections further revealed the presence of Hv1 in pancreatic islet ß-cells as assessed by colocalization with the marker insulin (Fig. 1B, a-d). Intriguingly, immunofluorescence on the dispersed mouse islet cells displayed the presence of Hv1 in insulin positive cells, which exhibited good merging with that of insulin (Fig. 1B, e-h), indicating that Hv1 localizes in insulin-containing granules. The anti-Hv1 monoclonal antibody used specifically recognizes Hv1, as shown by immunostaining (Fig. 1C). From these immunohistological and immunofluorescent observations at pancreatic tissues, isolated islets and ß-cell lines, we concluded that Hv1 is expressed in ß-cells, and preferentially localized in insulin-containing vesicles.

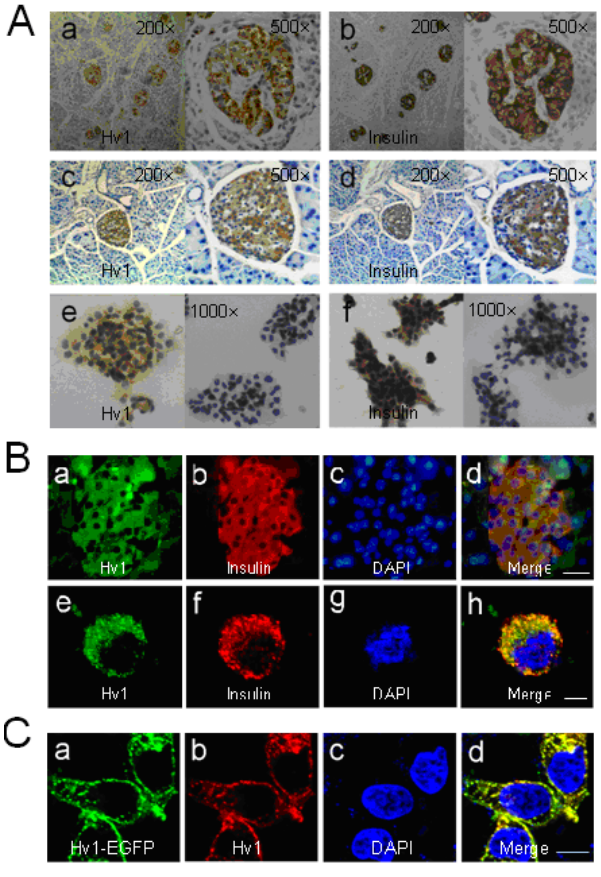

Fig. 1. Hv1 is present in human and mouse pancreatic islet β-cells.

(2) Knockdown of Hv1 inhibits glucose-induced insulin secretion

Hv1 markedly affects the glucose-induced insulin secretion in islets of Langerhans. The insulin secretion of the islets at a basal condition (2.8 mM glucose) was low at both control and Hv1-knockdown islets. Zn^{2+} a classical inhibitor of Hv1, inhibited glucose-induced insulin secretion by 52% at a concentration of 10 μM $ZnCl_2$ in islets. To confirm that the reduced insulin secretion in Hv1-knockdown isolated islets cells was caused by the reduction of endogenous proton channels, the isolated islets were exposed to gramicidin, a molecule that generates pores permeable to proton and other monovalent ions. In the presence of gramicidin the insulin secretion of Hv1-knockdown or $ZnCl_2$-blockage islets was almost restored by gramicidin to the control levels at 22.8 mM glucose.

Unsaturated fatty acids are well known to enhance H^+ current mediated by Hv1. Oleic acid (0.5 mM) and inoleic acid (0.5 mM) potentiated the insulin secretion 3.6-fold compared with that at the basal condition in isolated islets, similar to the effect mediated by glucose. This series of experiments confirmed that Hv1 is critical for insulin secretion in pancreatic islets and β-cell lines.

(3) Knockdown of Hv1 reduces direct depolarization-induced insulin secretion

Knockdown of Hv1 also affected direct depolarization-induced insulin secretion elicited by an increase of extracellular K^+. At a basal condition (in the presence of 3.6 mM KCl), the insulin secretion of the Hv1-deficient islets was slightly low, but in the presence of 60 mM KCl, the insulin secretion from the Hv1-deficient islets was significantly reduced by 38% compared with the control islets. 10 μM $ZnCl_2$ also inhibited K^+-induced the insulin secretion.

(4) Suppression of Hv1 affects cellular Ca^{2+} homeostasis

Glucose stimulates insulin secretion by induction of Ca^{2+}-dependent electrical activity that triggers exocytosis of the insulin granules. We monitored $[Ca^{2+}]_c$ in control and Hv1-silenced INS-1 (832/13) cells. At a basal condition the $[Ca^{2+}]_c$ levels in Hv1-silenced INS-1 (832/13) cells had a same pattern with that in the control cells. Following stimulation with 22.8 mM glucose, the $[Ca^{2+}]_c$ for Hv1-silenced cells lost thepeaks as displayed in the control cells, which was $[Ca^{2+}]_c$ oscillations due to bursts of Ca^{2+}-dependent action potentials. In comparison with glucose-stimulated Ca^{2+} responses, Ca^{2+} influx stimulated with 60 mM KCl was almost abrogated in Hv1-silenced cells. Thus, the reduction in insulin secretion by suppression of Hv1 must therefore involve in upstream of metabolic sensing, electrical activity, and $[Ca^{2+}]_c$ signaling.

(5) The effect of Zn^{2+} on the gating characteristics of Hv1

Zn^{2+} is a inhibitor of Hv1 and has been widely used for Hv1 function studdies. In this project, we also investigated the mechanism of Zn^{2+} on the gating regulation. There are two His residues in the C-terminal domain of Hv1, His^{244} and His^{266} (Fig. 2A). Zn^{2+}-induced precipitation of the C-terminal domain of Hv1 (wild type) depended on pH value and the mole ratios of the protein to Zn^{2+} (Fig. 2B). The mutants H244A and H266A behaved like the wild type (Fig. 2B). However, the Zn^{2+}-induced precipitation of the double mutant H244A/H266A did not occur at all from pH 5 to 8, indicating that Zn^{2+} interacts with both His^{244} and His^{266} residues.

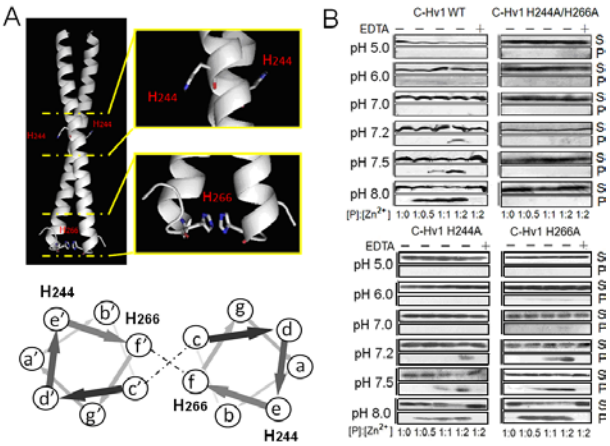

Fig. 2. Zn2+ binds to His residues in C-terminus of Hv1

To further confirm the binding sites of Zn^{2+} and the protonation sites, ITC experiments were carried out. The results showed that both His^{244} and His^{266} were Zn^{2+} binding sites and the two residues might be the protonation sites that regulates the channel gating.

（3－2）Impacts and Perspective

Here, we showed for the first time, that Hv1 is highly expressed in mouse and human pancreatic islet β-cells, and critical for insulin synthesis and secretion through regulating insulin secretory granule pH and membrane potential. Our study

sheds light on a new biological function of the voltage-gated proton channel Hv1. Furthermore, we found two sites for Zn^{2+} binding in the intracellular domain of Hv1, and the two sites might be the intracellular protonation sites that participate in regulating the channel gating.

［4］Publication lists

（the list of papers which contains the above results）

（1）Q. Zhao, S.J. Li, et al., The voltage-gated proton channel Hv1 is critical for insulin secretion in pancreatic islet ß-cells, under review.

（2）Q. Zhao, C. Li, S.J. Li, The pH-sensitive structure of the C-terminal domain of voltage-gated proton channel and the thermodynamic characteristics of Zn^{2+} binding to this domain, Biochem. Biophys. Res. Commun. 456 (2015) 207-212.