Protein interacting with C-kinase 1 modulates exocytosis and K_{ATP} conductance in pancreatic β cells

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Abstract. It has been previously identified that α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptors (AMPARs) are expressed in pancreatic β cells and regulate exocytosis and insulin release. It is known that protein interacting with C-kinase 1 (PICK1) regulates trafficking and synaptic targeting of AMPARs in the central nervous system. However, it is unknown whether PICK1 regulates glutamate-induced insulin release in β cells. The present study demonstrated that glutamate-induced exocytosis was increased in β cells derived from PICK1-knockout mice. In agreement with this result, adding PICK1 in β cells reduced glutamate-induced exocytosis, whereas adding EVKI, a peptide that interrupts the interaction between AMPARs and PICK1, increased the exocytosis of β cells with the application of glutamate. Furthermore, the conductance of ATP-sensitive potassium (K_{ATP}) channels was reduced in PICK1-knockout mice, which was reversed by the overexpression of PICK1. In addition, PICK1 application reduced voltage oscillation induced by the closure of K_{ATP}. Taken together, the results indicate that PICK1 regulates glutamate-induced exocytosis in β cells.

Introduction

Insulin is the major hormone regulating glucose metabolism and is predominantly stored in the secretory granules of pancreatic β cells (1-3). The classic pathway for insulin release is that, when blood glucose increases, glucose enters β cells through subtype 2 glucose transporters and changes the ratio of ATP/ADP. In addition, ATP-sensitive potassium channels (K_{ATP}) are important in regulating insulin release. The inhibition of K_{ATP} increases intracellular calcium and promotes the docking of insulin-containing granules (4). It has been previously demonstrated that α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) are expressed in mouse β cells and the activation of AMPARs leads to increased voltage oscillation and insulin release (4). The action of AMPARs is mediated by K_{ATP} because it is absent in Kir6.2 knockout mice (4).

Protein interacting with C-kinase (PICK1) is a PDZ and BAR domain-containing protein that emerges as a protein kinase C (PKC) binding protein (5). PICK1 binds to the C-terminal tail of AMPAR subunits GluA2/3 and regulates its trafficking and surface expression in the central nervous system (6-10). Disrupting their interaction inhibits GluA2 surface expression and long-term depression of synaptic transmission (11-13). It has been demonstrated that PICK1 is expressed in the pancreas (14). In addition, PICK1 knockout mice exhibit high blood glucose, insufficient insulin, and increased food and water intake, however lower body weight (15,16). Although PICK1 is essential to the formation and maturation of insulin granules (15,16), the function of PICK1 on AMPAR-mediated trafficking of insulin granules is unclear.

In the present work, it was investigated whether PICK1 regulates glutamate-induced exocytosis in β cells using whole-cell patch-clamp recordings in acute pancreatic tissue slices. It was identified that glutamate-induced exocytosis was increased in PICK1 knockout ($PICK1^{-/-}$) mice. In contrast, dialysis of fusion protein maltose binding protein (MBP)-PICK1 in β cells decreased the exocytosis induced by glutamate. Furthermore, it was demonstrated that PICK1 increased the conductance of K_{ATP} channels.

Materials and methods

Animals. All animal procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and all protocols were

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approved by the Animal Experimentation Ethics Committees of Ningxia Medical University (Yinchuan, China). Original breeding pairs of *PICK1*$^{-/-}$ mice were obtained from Professor Jun Xia (Hong Kong University of Science and Technology, Hong Kong, China) and maintained in the Experimental Animal Center of Ningxia Medical University. Mice were kept in temperature-controlled conditions under 12/12 h light/dark cycles with food and water available *ad libitum*. Wild type ICR mice were obtained from the Experimental Animal Center of Ningxia Medical University (Ningxia, China). A total of 24 male mice (age, 8-10 weeks; weight, 25-35 g) were used throughout the study. All drugs were from Sigma-Aldrich (Merck Millipore, Darmstadt, Germany), Tocris Cookson (Bristol, UK), or Ascent Scientific Ltd. (Bristol, UK) unless stated otherwise.

Pancreatic slice preparation. Pancreatic slices were prepared according to previous studies (4,17). The abdominal cavity of mice was opened and warm (37˚C) low-gelling agarose (1.9% wt/vol; Seaplaque GTG agarose; BioWhittaker Molecular Applications, Inc., Rockland, ME, USA) was injected into the distally clamped bile duct. The whole pancreas was immediately cooled with ice-cold extracellular solution (ECS, in mM): 125 NaCl, 2.5 KCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 2 Na-pyruvate, 0.5 ascorbic acid, 3 myo-inositol, 6 lactic acid, 1 $MgCl_2$ and 2 $CaCl_2$, adjusted to pH 7.3 and oxygenated with 95% O_2/5% CO_2, 300±10 mOsm/kg. The pancreas was hardened agarose was extracted, placed in a small dish filled with warm agarose, and cooled rapidly on ice. Four small cubes were cut from the agarose-embedded pancreatic tissue and glued onto the sample plate of a vibratome (VT1000S; Leica Microsystems GmbH, Wetzlar, Germany). Slices (140 μm) were sectioned at a speed of 0.05 mm/s and 70 Hz in ice-cold ECS bubbled with 95% O_2/5% CO_2. Slices were kept in ice-cold ECS for at least 1 h prior to use.

Electrophysiology. Slices were placed in a submerged chamber and perfused with ECS (30˚C) at 1.5 ml/min. Cells were visualized under an upright microscope (Zeiss Axioskop 2 FS; Carl Zeiss AG, Oberkochen, Germany) and a mounted with a Cohu CCD camera (Cohu, Inc., Poway, CA, USA) with 5X digital amplification. β cells from second or third layers in selected islets were used for whole-cell recordings. The recording pipettes were pulled on an electrode puller (P-97; Sutter Instrument Co., Novato, CA, USA) and had resistances of 2-4 MΩ. To measure the glutamate currents, electrodes were filled with a solution containing 125 mM CsCl, 40 mM HEPES, 2 mM $MgCl_2$ and 20 mM tetraethylammonium-Cl (pH 7.2 with CsOH). To measure voltage oscillation and K_{ATP} conductance, electrodes were filled with a solution containing: 150 mM KCl, 10 mM HEPES, 2 mM $MgCl_2$, 0.05 mM EGTA (pH 7.2 with KOH). Currents were filtered at 3 kHz and digitized at 10 kHz using an EPC10 amplifier (HEKA Elektronik Dr. Schulze GmbH, Lambrecht/Pfalz, Germany). MPS-2 multichannel microperfusion (MPS-2; INBIO, Shanghai, China) was used to locally puff solutions onto cells. Each injector delivered solution at a flow rate of 0.25 ml/min. The amplitude of the equilibrium response was the mean value of data points measured the final second prior to the termination of drug application.

Exocytosis detection. The capacitance change induced by depolarization pulses (18-20) was used to measure the exocytosis of β cells. A 20 mV peak-to-peak 800 Hz sine wave was added to the holding potential and 10 cycles were averaged for each data point.

K_{ATP} channel conductance. K_{ATP} channel conductance (G_{KATP}) was measured according to a previous study (4). Whole-cell patch-clamp with K^+-based 0 ATP pipette solution was made to allow dialysis of intracellular ATP. After the whole-cell configuration, ramp stimuli (-100 mV to-40 mV at 0.6 mV/msec steps) were applied at 1 Hz. The dominant current component between -100 and -40 mV ran through tolbutamide-sensitive K_{ATP} channels. At membrane potentials exceeding-30 mV, voltage-dependent K^+ channels were activated (21). Mean amplitudes of current in the first and last 10 msec were acquired in an individual ramp. The increments of current between these two durations (ΔI) were calculated as current values of the ramp. The increment of voltage between first and last 10 msec (ΔV) was measured as 54 mV. K_{ATP} channel conductance was thereby calculated by Ohm's law (G_{KATP}=ΔI/54 mV) and depicted as one data point.

Data analysis. The statistical significance of the differences between groups was determined using one-way analysis of variance followed by a post hoc Tukey's test for multiple comparisons. $P<0.05$ was considered to indicate a statistically significant difference. Data in the text and figures are presented as the mean ± standard error. Statistical analysis was performed using SPSS software version 16.0 (SPSS, Inc., Chicago, IL, USA).

Results

PICK1 modulates exocytosis of β cells. It was previously demonstrated that a 500-msec depolarizing pulse leads to a calcium influx and stimulates exocytosis and AMPARs participate in the exocytosis of β cells in the bath solution containing 3 mM glucose (4). Using the same method, the effect of PICK1 on β cell exocytosis was examined. To achieve this, a fusion protein, MBP-PICK1, was synthesized and dialyzed into β cells through patch pipettes. β cells were voltage-clamped at -80 mV and depolarized to 0 mV for 500 msec in order to induce exocytosis. Capacitance changes were defined as the difference between capacitances prior and subsequent to depolarization. Responses during depolarization were omitted (dashed line) because of irregular spikes (22). The averaged values of increased normalized capacitance were 12.1±1.5 fF/pF (wild type, n=15), 11.9±1.6 fF/pF (PICK1, n=16), 16.5±1.4 fF/pF (glutamate, n=15), 5.0±1.8 fF/pF (glutamate + PICK1, n=17) (Fig. 1A). The effect of EVKI (100 μM), a peptide that interferes with the interaction between GluR2 and PICK1 (23), was observed on the basal and depolarization-induced exocytosis. Increased normalized capacitance was 10.5±1.2 fF/pF (n=16) in the basal group and 18.6±2.0 fF/pF (n=16) in the EVKI + glutamate group (Fig. 1B), indicating that EVKI increases glutamate-evoked exocytosis. Subsequently, the effect of the application of PICK1 + EVKI or PICK1 + EVKI + glutamate were examined and no significantly different change in glutamate-induced exocytosis

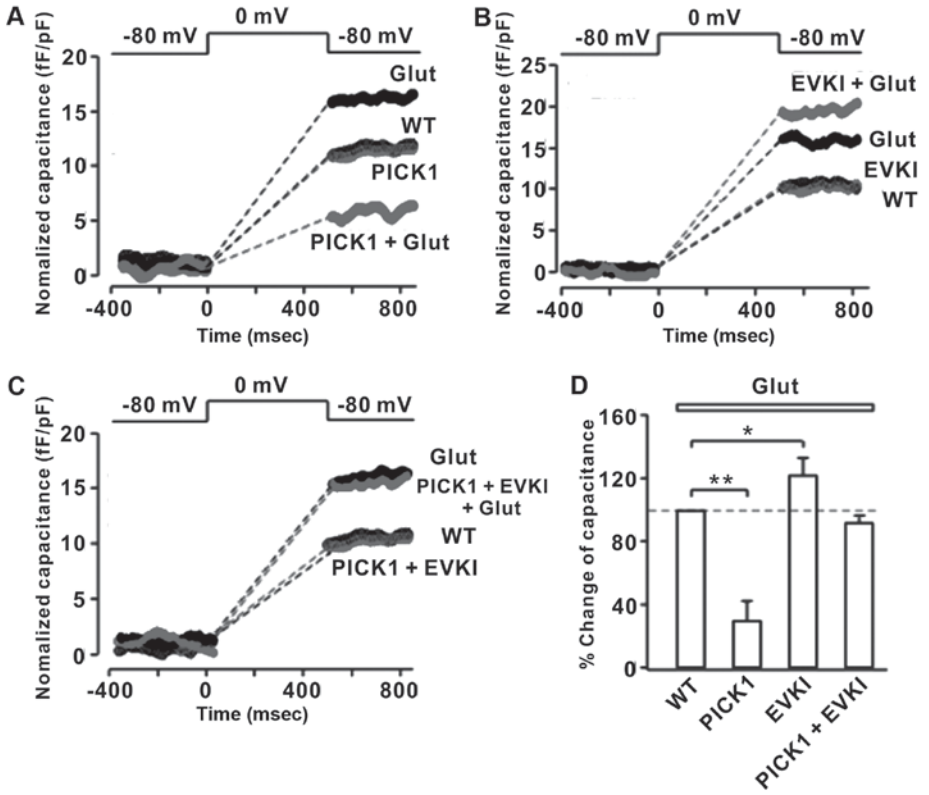

Figure 1. PICK1 inhibits Glut-induced exocytosis. (A) Representative exocytosis responses from WT β cells with or without internal MBP-PICK1. WT, t=2 min; PICK1, WT β cell loaded with MBP-PICK1, t=2 min; Glut, WT β cell with Glut stimulation, t=8 min; PICK1 + Glut, WT β cell loaded with MBP-PICK1 and stimulated with Glut, t=8 min. (B) Representative exocytosis responses from WT β cells with or without internal EVKI. WT, t=2 min; EVKI, WT β cell loaded with EVKI, t=2 min; Glut, WT β cell with Glut stimulation, t=8 min; EVKI + Glut, WT β cell loaded with EVKI and Glut stimulation, t=8 min. (C) Representative exocytosis responses from WT β cells with or without internal MBP-PICK1 and EVKI. WT, t=2 min; PICK1 + EVKI, WT β cell loaded with MBP-PICK1 and EVKI, t=2 min; Glut, WT β cell with Glut stimulation, t=8 min; PICK1 + EVKI + Glut, WT β cell loaded with MBP-PICK1 and EVKI and stimulated with Glut, t=8 min. (D) Percentages of capacitance were 29±13% (PICK1, n=20), 121±11% (EVKI, n=22) and 98±5% (PICK1 + EVKI, n=19). *P<0.05, **P<0.01. PICK1, protein interacting with C-kinase 1; Glut, glutamate; WT, wild type; MBP, maltose binding protein.

was observed between these two groups (PICK1 + EVKI, 10.1±0.9 fF/pF, n=17; PICK1 + EVKI + glutamate, 10.3±1.0 fF/pF, n=17) (Fig. 1C). Together, the results demonstrated that PICK1 decreased but EVKI increased the exocytosis of β cells (Fig. 1D), indicating that PICK1 prevents AMPAR-mediated β cell exocytosis.

Whether exocytosis is changed in β cells from PICK1$^{-/-}$ mice was further examined. It was identified that normalized capacitance was increased to 19.9±2.4 fF/pF (n=17) in PICK1$^{-/-}$ mice (Fig. 2A) compared with wild type mice, indicating that glutamate-induced exocytosis is increased in PICK1$^{-/-}$ mouse β cells.

PICK1 modulates K_{ATP} conductance. A previous study indicated that AMPAR activation increases cytosolic cyclic guanosine monophosphate (cGMP) and inhibits K_{ATP} channels (4). In addition, inhibition of K_{ATP} increases $[Ca^{2+}]_i$ and promotes the docking of insulin-containing granules (4). Therefore, whether PICK1 also affects K_{ATP} was investigated. A K^+-based ATP-free pipette solution was used in whole-cell recordings to measure the conductance of K_{ATP} (4,24,25). Subsequent to establishing whole-cell configuration, K_{ATP} conductance rapidly increased to a peak (Fig. 3A) within 2 min. The mean maximum value was 3.9±0.2 nS/pF (Fig. 3B; n=12). PICK1 treatment increased the peak amplitude to 4.6±0.2 nS/pF (n=11, P<0.05) while peak amplitude declined to 3.1±0.2 nS/pF in PICK1$^{-/-}$ β cells (n=11, P<0.05). These data suggest that PICK1 modulates β cell exocytosis through increasing K_{ATP} channel conductance.

PICK1 modulates K_{ATP}-dependent voltage oscillation. Closure of K_{ATP} by cytosolic ATP induces a typical spontaneous burst-like membrane voltage oscillation (21), which is a determining factor for insulin secretion (21,26). The effect of PICK1 on K_{ATP}-dependent voltage oscillation was therefore examined. β cells were perfused with a background solution containing 11 mM glucose, which elicited stable bursts that commonly appeared within 30 min subsequent to the formation of the whole-cell clamp, and reliably lasted as long as the whole-cell recording continued. Following the baseline recordings, PICK1 was allowed to perfuse into β cells through patch pipettes to assess its effect on the oscillation (Fig. 4A). The average amplitude and duration of spikes were 18.5±0.9 mV and 13.1±0.5 sec (n=15) in the control, while PICK1 attenuated burst amplitude to 12.5±0.8 mV (n=16) and burst duration to 7.8±0.4 sec (n=16) (Fig. 4B). In agreement with Fig. 2, these data clearly indicated suggest that PICK1 modulates K_{ATP} channel.

Discussion

In the present study, it was identified that glutamate-induced exocytosis in β cells was reduced by PICK1, however was

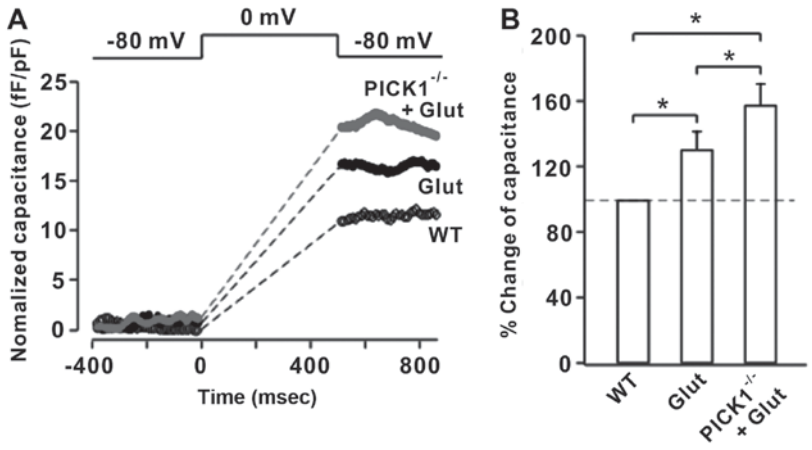

Figure 2. Glut-induced exocytosis is enhanced in PICK1$^{-/-}$ mice. (A) Representative exocytosis responses from one WT β cell and one PICK1$^{-/-}$ β cell. WT, t=2 min; Glut, WT β cell with Glut stimulation, t=8 min; PICK1$^{-/-}$ + Glut, PICK1$^{-/-}$ β cell with Glut stimulation, t=8 min. (B) Statistical percentage capacitance changes were 131±11% (Glut, n=20) and 159±14% (PICK1$^{-/-}$ + Glut, n=20). *P<0.05. Glut, glutamate; PICK1, protein interacting with C-kinase 1; WT, wild type.

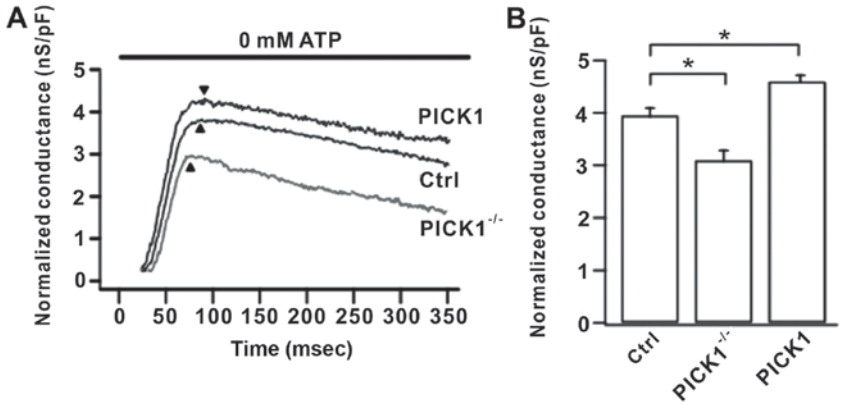

Figure 3. PICK1 modulate K_{ATP} channel conductance. (A) K_{ATP} conductance measured from one cell internal dialysis with 0 mM ATP in the pipette. K_{ATP} conductance in extracellular solution (Ctrl, n=18), PICK1 (PICK1, n=19) and β cells of PICK1 knock-out mice (PICK1$^{-/-}$, n=20). Black triangles indicate the peak of each response. The short duration between rupture (0 msec) and the first data point was used for membrane capacitance compensation. Note that the difference the in times to maximum were suggested to be due to different cell sizes and pipette tips, which affected the diffusion rate (13). (B) Statistics of maximal K_{ATP} conductance of β cells were 3.9±0.2 nS/pF (Ctrl, n=12), 3.1±0.2 nS/pF (PICK1, n=11) and 4.6±0.2 nS/pF (PICK1$^{-/-}$, n=11). *P<0.05. PICK1, protein interacting with C-kinase 1; ATP, adenosine triphosphate; Ctrl, control.

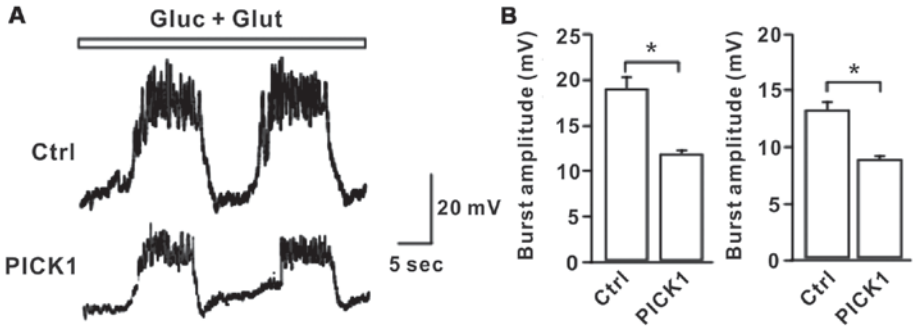

Figure 4. PICK1 inhibits Glut-induced burst oscillation. (A) Representative adjacent pairs of spikes random selected from Gluc + Glut (600 μM) and Gluc + Glut + MBP-PICK1 (50 ng/ml) groups. (B) Statistics of burst amplitude and duration on bath application of Gluc + Glut were 18.5±0.9 mV and 13.1±0.5 sec (Ctrl, n=10) and 12.5±0.8 mV and 7.8±0.4 sec (PICK1, n=11). *P<0.05. PICK1, protein interacting with C-kinase 1; Glut, glutamate; Gluc, glucose; Ctrl, control.

increased by knocking out PICK1. EVKI and MBP-PICK1 significantly altered glutamate-induced exocytosis. Together, it was suggested that PICK1 inhibits β cell exocytosis through AMPARs.

Glutamate fails to trigger insulin release with low-level glucose however increases insulin release in the condition of high glucose (27). It was reported that glutamate stimulates glucagon secretion through activating metabotropic and

ionotropic glutamate receptors in β cells (28,29). It was previously demonstrated that AMPARs are expressed in mouse β cells and glutamate produces cGMP and decreases the conductance of K_{ATP} (4). Inhibition of K_{ATP} controls the gating of VGCCs and increases extracellular Ca^{2+} influx (30). When $[Ca^{2+}]_i$ is high enough to promote the docking of insulin-containing granules, exocytosis and insulin release are thereby enhanced with a high driving force (depolarization or high glucose stimulation) (31).

The best characterized function of PICK1 is that it regulates trafficking of the AMPAR subunit GluA2 during long-term depression and long-term potentiation. Associated with a previous study demonstrating that PICK1 is expressed in β cells (32), the present study demonstrated that PICK1 functions in β cell exocytosis in an AMPAR-dependent manner, due to the fact that PICK1 had no effect on basal exocytosis however significantly altered glutamate-induced exocytosis. A previous study indicated that the total insulin level is reduced and proinsulin is increased in PICK1-deficient β cells (15). In addition, PICK1 deficiency impairs immature secretory vesicle storage of insulin budding from the trans-Golgi network and leads to decreased glucose tolerance (16). While these results suggest that PICK1 is a key regulator of the formation and maturation of insulin granules, the present study indicates a different role of PICK1 in the condition of excessive glutamate.

AMPARs are additionally identified in α cells that are essential for glucagon release (27). Reducing glucose concentration results in the release of glutamate from α cells and enhances glucagon release through an autocrine action (27). This glutamate autocrine feedback loop endows α cells with the ability to potentiate its own secretory activity (33). It will be of interest to explore whether PICK1 also acts on this autocrine signaling in α cells.

The burst-like membrane voltage oscillation that is induced by closure of K_{ATP} channel was measured, and was indicated to be a determining factor for insulin secretion (21,26). The data demonstrated that PICK1 downregulated voltage oscillation induced by glucose and glutamate and modulates K_{ATP}. Future studies are required in order to explore the association between PICK1 and K_{ATP}.

Acknowledgements

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