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Two populations of pancreatic islet α-cells displaying distinct Ca^{2+} channel properties ☆

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Abstract

In low or absence of glucose, α-cells generate rhythmic action potentials and secrete glucagon. α-Cell T-type Ca^{2+} channels are believed to be pacemaker channels, which are expected to open near the resting membrane potential (around −60 mV) to initiate a small depolarization. A previous publication, however, showed that α-cell T-type Ca^{2+} channels have an activation threshold of −40 mV, which does not appear to fulfill their role as pacemakers. In this work, we investigated the Ca^{2+} channel characteristics in α-cells of mouse-insulin-promoter green-fluorescent-protein (MIP-GFP) mouse. The β-cells of MIP-GFP were conveniently distinguished as green cells, while immunostaining indicated that the majority of non-green cells were α-cells. We found that majority of α-cells possessed T-type Ca^{2+} channels having an activation threshold of −40 mV; these cells also had high-voltage-activated (HVA) Ca^{2+} channels (activation threshold of −20 mV). A novel finding here is that a minority of α-cells had T-type Ca^{2+} channels with an activation threshold of −60 mV. This minor population of α-cells was, surprisingly, devoid of HVA Ca^{2+} channels. We suggest that this α-cell subpopulation may act as pacemaker cells in low or absence of glucose.

Keywords: Islet α-cells; T-type Ca^{2+}channels; High-voltage-activated Ca^{2+}channels; Pacemaker

Pancreatic islet β- and α-cells secrete insulin and glucagon, respectively, which reciprocally regulate glucose homeostasis [1,2]. Stimulation of β-cell insulin secretion by high glucose is via glucose metabolism and ATP production, subsequently leading to K_{ATP} channel inhibition, membrane depolarization, and exocytosis [3]. By contrast, α-cells secrete glucagon in low or absence of glucose and cease to secrete glucagon in high glucose [2]. In low glucose, it is proposed that T-type Ca^{2+} channels act as pacemaker channels [4,5]. The small depolarization thus initiated activates Na^{+} channels and subsequently triggers high-voltage-activated (HVA) Ca^{2+} channel opening (L- and N-type), eventually culminating in exocytosis [4,5]. How high glucose silences α-cell secretion has remained poorly understood and controversial [5–9]. Substantial evidence has hitherto accumulated to favor the view that β-cell secretory products (GABA, insulin, and Zn^{2+}) are strong inhibitors of glucagon secretion [10–14].

For the T-type Ca^{2+} channels to act as pacemakers for the α-cells, and to open near the resting membrane potential (~60 mV; mouse α-cells, Refs. [6,15]; rat α-cells, Refs. [13,16]), they are expected to have an activation threshold close to −60 mV. However, there have only been a few reports characterizing Ca^{2+} channels in α-cells (all in mouse, Refs. [5,6,17,18]). These works either showed the absence of T-type Ca^{2+} channels in α-cells [6,18], or that the α-cell T-type Ca^{2+} channels have an activation threshold of

☆ *Abbreviations:* MIP-GFP, mouse insulin promoter-green fluorescent protein; V_H, holding potential; pF, picoFarad; HVA, high-voltage-activated; LVA, low-voltage-activated; VDCC, voltage-dependent Ca^{2+} channel; TTX, tetrodotoxin; *I–V*, current–voltage relationship.

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−40 mV [5,17], which does not appear to fulfill their role as pacemakers. In this work, we employed the MIP-GFP mouse where GFP is expressed in β-cells, and where the majority of non-green cells (~80%) are α-cells [14,17], thereby enabling us to examine α-cells in much higher throughput and reliability. Here, we found that majority of α-cells possessed T-type Ca^{2+} channels having an activation threshold of −40 mV; these cells also had HVA Ca^{2+} channels. Interestingly, we also found that a minority of α-cells had T-type Ca^{2+} channels with an activation threshold of −60 mV. This minor population of α-cells, surprisingly, lacked HVA Ca^{2+} channels. We suggest that this α-cell subpopulation may act as pacemaker cells in low or absence of glucose.

Materials and methods

Materials. Antibody against glucagon was purchased from Sigma (St. Louis, MO). Tetraethylammonium–Cl (TEA) and Mg-ATP were from Sigma. Tetrodotoxin (TTX) was from Alomone Labs, Jerusalem, Israel.

Islet isolation. MIP-GFP mouse pancreatic islet cells were isolated by collagenase digestion as described previously [14,17,19]. Islets were dispersed to single cells with 0.05% trypsin (Sigma) in Ca^{2+}- and Mg^{2+}-free PBS. Islet cells were plated on glass coverslips in 35-mm dishes and cultured in RPMI 1640 medium containing 1 mM pyruvate, 11 mM glucose, 0.2% $NaHCO_3$, 10% fetal bovine serum, 10 mM HEPES, and 100 U/ml penicillin G sodium, 100 μg/ml streptomycin sulfate (Invitrogen). Islet cells were cultured overnight before electrophysiological recordings.

Confocal immunofluorescence microscopy. Laser confocal immunofluorescence microscopy was performed as previously described [14,17]. Dispersed pancreatic islet cells were plated on glass coverslips coated with 0.01% poly-L-lysine. After 2 h, the cells were fixed in 2% formaldehyde for 30 min, then treated with 5% goat serum and 0.1% saponin for 1 h, and finally immunolabeled with mouse monoclonal anti-glucagon (1:2000) for 2 h. The coverslips were rinsed with 0.1% saponin in phosphate-buffered saline and then incubated with Texas red-labeled secondary antibody for 1 h. After rinsing once more, coverslips were mounted on slides in a fading retarder (0.1% *p*-phenylenediamine in glycerol) and examined using a laser scanning confocal imaging system (LSM 510, Zeiss, Germany).

Recording of Ca^{2+} currents. Mouse islet α-cells were voltage-clamped in the whole-cell configuration using an EPC-9 amplifier and Pulse software (HEKA Electronik, Lambrecht, Germany) as we previously described [14,17]. As already described, the green cells of this MIP-GFP mouse are β-cells, while non-green cells are mostly α-cells with the rest being δ-cells. To study the α-cells, non-green cells were chosen and only those having T-type Ca^{2+} currents were considered α-cells [4,5,17]. T-type Ca^{2+} currents are absent in δ-cells [4,15]. Pipettes tip resistances ranged from 3 to 5 MΩ when filled with intracellular solutions. The intracellular solution for Ca^{2+} current measurement contained (mM): 120 CsCl; 20 TEA–Cl; 1 $MgCl_2$; 1 EGTA; 10 HEPES, and 5 MgATP (pH 7.25, adjusted with CsOH). The bath solution for Ca^{2+} current measurement contained (mM): 140 NaCl; 4 KCl; 1 $MgCl_2$; 10 $CaCl_2$; 2 D-glucose; 10 HEPES; 20 TEA–Cl; 0.01 TTX (pH 7.4, adjusted with NaOH). TTX was used to block Na^+ currents. After a whole-cell configuration was established, the cell was held at either −100 or −50 mV and stimulated by a series of pulses (from −70 to +70 mV at 10 mV increments, 500 ms duration). All experiments were performed at room temperature (~22 °C).

Results

The majority of non-green cells are α-cells

Fig. 1A shows a brightfield image of dispersed islet cells. The white arrows point to the β-cells, which were green in the confocal image as they expressed GFP (Fig. 1B). The black arrows in Fig. 1A indicate α-cells, which were labeled with glucagon antibody and appeared in red (Fig. 1C). Fig. 1D shows another brightfield image of dispersed islet cells. The white arrows point to the β-cells (green in Fig. 1E). The black arrows in Fig. 1D indicate α-cells (red in Fig. 1F). The broken arrow in Fig. 1D points to a non-green cell which was not labeled with glucagon antibody. This cell is likely a δ-cell. Therefore, these results are consistent with our previous observation that the majority of non-green cells are α-cells [14,17].

Characterization of single α-cell VDCC

Previous works have shown that mouse β- and δ-cells possess only HVA Ca^{2+} channels, while α-cells have both LVA- and HVA-Ca^{2+} channels [4,5,15,17]. In this study, therefore, only those non-green cells having T-type Ca^{2+} currents were considered α-cells. Most of the cells (9 out of 12 examined) displayed Ca^{2+} channel features shown in Fig. 2. When the α-cell was held at −100 mV and depolarized to different potentials, Ca^{2+} currents could readily be triggered at −40 mV and were transient (fast activation and inactivation), characteristic of LVA Ca^{2+} channels or the T-type VDCC (Fig. 2A, left). Ca^{2+} currents activated at stronger depolarization (−20 or −10 mV) began to show, in addition to the transient current, a sustained component, indicative of HVA Ca^{2+} channels. When V_H was at −50 mV, which would readily inactivate LVA Ca^{2+} channels, depolarization expectedly only triggered the slow-inactivating HVA Ca^{2+} currents (Fig. 2A, middle). The activation threshold for these HVA Ca^{2+} currents was about −20 mV. The difference between the Ca^{2+} currents obtained at the two V_H gives the LVA Ca^{2+} currents (Fig. 2A, right). The results are summarized in the *I–V* curves shown in Fig. 2B ($n = 9$). Thus, the total currents (V_H at −100 mV) were the sum of HVA (V_H at −50 mV) and LVA Ca^{2+}currents (the difference currents). The HVA and LVA Ca^{2+} currents peaked at +10 mV and −20 mV, respectively.

The LVA Ca^{2+} channels reported before [5,17] and here (see above, 9 out of 12 cells) had a threshold of −40 mV, which does not appear to be low enough to permit pacemaking activities around the resting potential of −60 mV [6,13,15,16]. Interestingly, in 3 out of 12 cells we examined, the α-cell showed some "anomaly" in Ca^{2+} channel characteristics. In these cells, rapidly activating and inactivating T-type Ca^{2+} currents were triggered at as low as −60 mV (Fig. 3A, right, difference currents). The T-type Ca^{2+} currents peaked at around −50 to −40 mV. HVA currents, surprisingly, were not observed at all, even at voltages as depolarized as 0 mV (Fig. 3A, middle). Intriguingly, some outward currents were triggered at voltages beginning at 0 mV or higher (Fig. 3A, left and right); these outward currents were substantially inactivated at $V_H = -50$ mV (Fig. 3A, middle). The nature of these outward currents is unknown, but they are unlikely K^+ currents since K^+ in the pipette solution was completely replaced by Cs^+, a

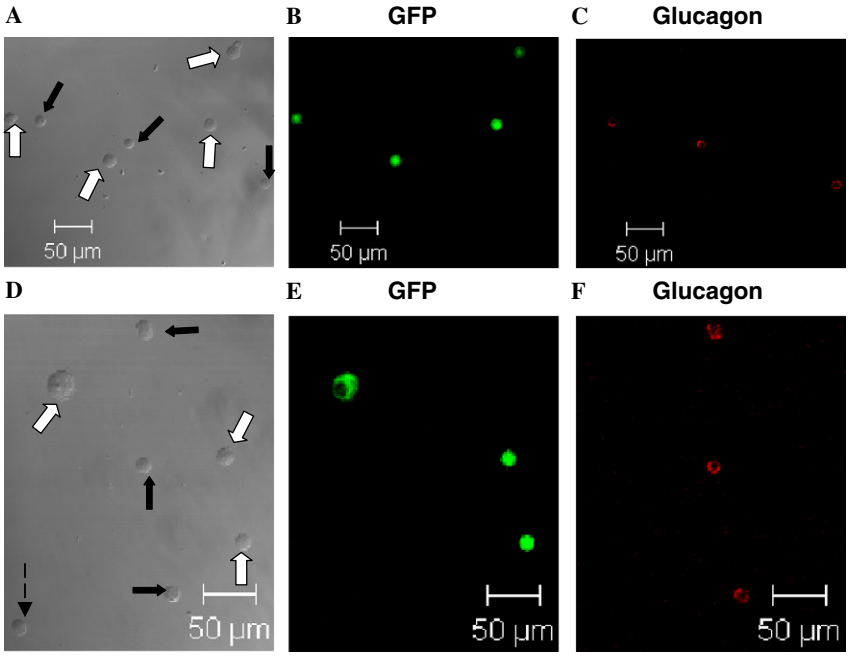

Fig. 1. Visual identification of islet cells. (A) Brightfield image of islet cells. α- and β-cells were labeled by black and white arrows, respectively. (B) β-Cells expressing GFP. (C) α-Cells with immunofluorescence labeling. (D) Another brightfield image of islet cells. α- and β-cells were labeled by black and white arrows, respectively. The cell indicated by the broken arrow is likely a δ-cell. (E) β-Cells expressing GFP. (F) α-Cells with immunofluorescence labeling. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

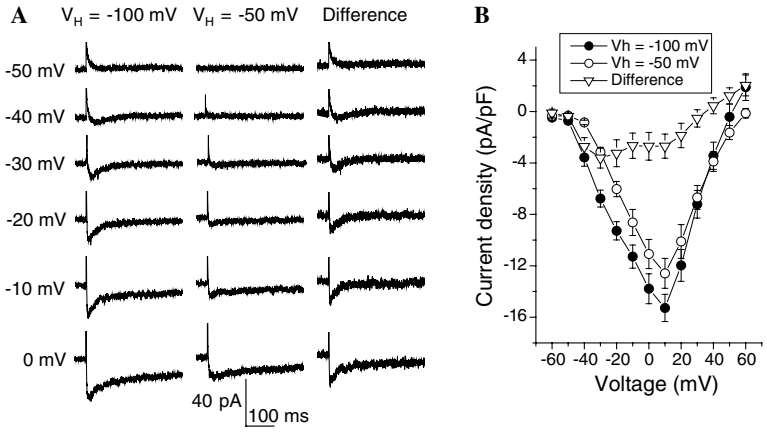

Fig. 2. VDCC in one group of α-cells having both LVA and HVA Ca^{2+} channels. (A) Representative traces of Ca^{2+} currents of α-cells stimulated at different voltages and at different V_H. The difference between the currents obtained at $V_H = -50$ mV and -100 mV yields the difference currents (T-type Ca^{2+} currents). (B) I–V plots at different V_H. All values are means ± SEM of 9 cells.

K^+ channel blocker. The results are summarized in the I–V curves shown in Fig. 3B ($n = 3$). Note that there were only T-type Ca^{2+} currents in these cells and that the reversal potential for the T-type Ca^{2+} currents was unusually negative (between -10 and 0 mV). This may be due to the presence of the outward currents of unknown nature, which began to activate at around 0 mV.

Discussion

In order for the T-type Ca^{2+} channels to act as pacemaking channels for the α-cells, and to open near the α-cell resting membrane potential (~60 mV; Refs. [6,13,15,16]), their activation threshold has to be close to -60 mV. However, α-cell T-type Ca^{2+} channels have been reported to have an activation threshold of -40 mV [5,17]. This throws doubt on whether these α-cells are truly pacemaker cells. In this work, we found that the majority of α-cells (75%) indeed possessed T-type Ca^{2+} channels having an activation threshold of -40 mV; these cells also had HVA Ca^{2+} channels. A novel finding here is that a minority of α-cells (25%) have T-type Ca^{2+} channels with an activation threshold of -60 mV. These T-type Ca^{2+} channels had the typical fast activation and inactivation

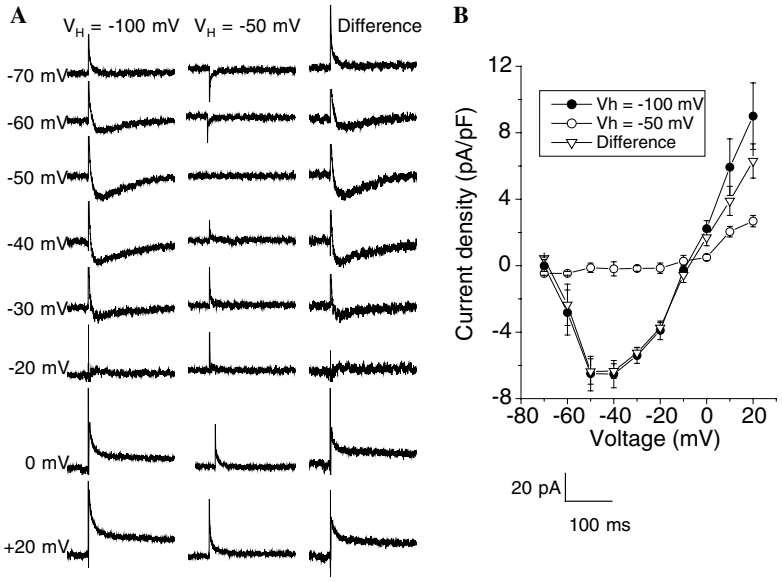

Fig. 3. VDCC in one group of α-cells having only LVA Ca^{2+} channels. (A) Representative traces of Ca^{2+} currents of α-cells stimulated at different voltages and at different V_H. The difference between the currents obtained at $V_H = -50$ mV and -100 mV yields the difference currents (T-type Ca^{2+} currents). (B) *I–V* plots at different V_H. All values are means ± SEM of 3 cells.

kinetics. Surprisingly, this minor population of α-cells was devoid of HVA Ca^{2+} channels.

This minor population of α-cells, having T-type Ca^{2+} channels with -60 mV activation threshold, is therefore strongly implicated in pacemaking the oscillating membrane potential under low glucose condition. As HVA, but not LVA, Ca^{2+} channels have been known to participate in α-cell exocytosis [20], the lack of HVA Ca^{2+} channels in this minor subpopulation may suggest that they are devoid of glucagon release capacity. The majority of α-cells, having HVA Ca^{2+} channels, are expected to have full exocytotic capacity. It is therefore tempting to speculate that a minority of α-cells function as pure pacemakers, which initiates rhythmic action potential firing and hence glucagon secretion in the rest (majority) of the α-cells. The electrical coupling may be through gap junctions between individual α-cells of the islet. This proposal certainly awaits verification in future experimentation. In type I or advanced type II diabetes, α-cell glucagon secretion is severely impaired. It would be of interest to know in such situations, whether α-cell Ca^{2+} channel characteristics would be altered, or whether the distribution of α-cell subpopulations would be affected.

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