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Electrophysiological characterizations of pancreatic islet cells

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Human Islet Research Network

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ABSTRACT

Hormone release from pancreatic cells, for example glucagon from α-cells and insulin from β-cells, is controlled by the cells' membrane potentials (Vm), which are in turn finely controlled by multiple classes of ion channels, transporters, and pumps. The perforated whole-cell patch-clamp method represents a direct and well-established way to monitor Vm in individual cells and also to investigate ionic currents (Im) that contribute to determination of Vm. Here a step-by-step guide to obtain various patch-clamp measurements from pancreatic cells in isolated intact human islets is provided. The method requires a standard electrophysiological station and β escin is used as the perforating agent. Select exemplar current-clamp Vm results and voltage-clamp Im results are provide. The electrophysiological characteristics may be used to distinguish pancreatic cell types, for example α-cells vs. β-cells in real time.

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EXTERNAL LINK

https://hpap.pmacs.upenn.edu/assets/workflow/Islet%20Physiology%20Studies/Intracellular%20Calcium%20Flux%20and%20Electrophysiology.pdf

PROTOCOL CITATION

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MATERIALS TEXT

Reagents/Buffers/Materials Needed for Experiments

1. A complete electrically quiet electrophysiological setup
a. A vibration isolate table
b. An inverted (or an upright) microscope
c. An inline-perfusion solution heater
d. A microscope stage heater
e. A ow-speed peristaltic pump
f. A vacuum source (a house vacuum line or a vacuum pump) and a waste flask
g. A micromanipulator
h. A patch-clamp amplifier with its head stage assembly mounted on the aforementioned micromanipulator
i. An analog-to-digital/digital-to-analog converter (if not integrated into the patch-clamp amplifier)
j. A data acquisition program
k. A recording chamber with an Ag/AgCl pellet ground wire

2. pipette puller

3. Electrode tip polisher

4. Sylgard 184 elastomer or dental wax to coat electrodes to reduce capacitance

5. Patch-clamp glass

6. A small bath sonicator

7. A dissection microscope

8. A diamond glass cutting pen and a pair of tweezers

9. Recording solutions
a. No glucose external solution
130 mM NaCl, 4mM KCl, 2 $CaCl_2$, 2 $MgCl_2$, 30 mM mannitol, 10 mM HEPES, pH **7.4** with *N-methyl-D*-glucamine at **35 °C**

b. 5 mM glucose external solution
130 mM NaCl, 4mM KCl, 2 $CaCl_2$, 2 $MgCl_2$, 15 mM mannitol, 10 mM HEPES, pH **7.4** with *N-methyl-D*-glucamine at **35 °C**

c. 25 mM glucose external solution
130 mM NaCl, 4mM KCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 mM HEPES, pH **7.4** with *N-methyl-D*-glucamine at **35 °C**

d. Sulfate internal solution
76 K_2SO_4 mM, 10 mM KCl, 10 mM NaCl, 6 $MgCl_2$, 30 mM mannitol, 10 mM HEPES, pH **7.4** with *N-methyl-D*-glucamine at **35 °C**

Note:

Based on the Ca^{2+} chelating abilities of sulfate, the free Ca^{2+} concentration is estimated to be in the low µM range and the free Mg^{2+} concentration should be about 2 mM

10. β-escin (8mM in water)
The final concentration is 8 µM; diluted with the internal solution before each recording session and sonicate

Procedure

1 Sonicate 8 µM escin diluted with the internal solution in the bath sonicator for > 10 min
4 µM escin is sometimes sufficient

2 Start the perfusion of the recording chamber so that the chamber solution is at **35 °C** ; the perfusion is continuous

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3 Take a culture dish with a coverslip with pancreatic islets (or pancreatic islet cells) using the dissection microscope

4 Cut out a small section, with an islet (or cells), using a diamond pen, and transfer the coverslip piece to the recording chamber filled with the desired recording solution (e.g., 5 mM glucose)

5 Equilibrate the islet (or cells) in the chamber for > 10 min

6 Fill the tip of a polished, sylgard- or wax-coated patch electrode with the internal recording without escin and back fill with the recording with escin

7 The input resistance of the electrode should be 3 to 6 Mohms, depending on the types of the data required; lower for voltage-clamp experiments and higher for current-clamp experiments.

8 Form a seal with a few Gohms in resistance

9 Change the holding voltage to —70 mV (in the whole-cell mode convention) and apply small short square pulses (—10 mV in size from the holding voltage and 20 ms in duration) to monitor the resistance and capacitance

10 Within 5 to 10 min, adequate perforated whole-cell access should be achieved

11 Apply short small square pulses in the voltage-clamp mode (—10 mV in size from the holding voltage and 20 ms in duration) to measure the input resistance and capacitance

12 Compensate the whole-cell capacitance and series resistance

13 Measure membrane potential (V_m) in the current-clamp mode; change the external solutions if desired

14 13 Measure membrane potential (I_m) in the voltage-clamp mode; change the external solutions if desired

Data analysis

15 1. Export data to IgorPro (Wavemerics) for data plotting and analysis

2. Data visualization
a. Current-clamp V_m measurement example (from a cell in an intact islet)

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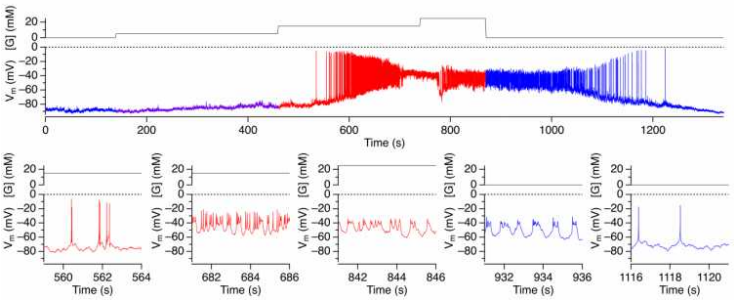
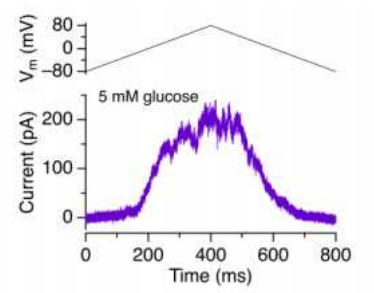
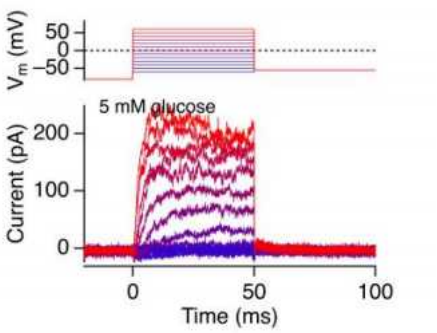


b. Ramp voltage-clamp I_m measurement example (from a sorted β cell)



c. Step voltage-clamp I_m measurement example (from a sorted β cell)



3. Other custom analysis

a. Igor scripts may be written for custom and automated analysis procedures as needed

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