Human islet electrophysiology

The Hoshi Laboratory

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I. **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 low-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, 4 mM 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, 4 mM 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, 4 mM 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

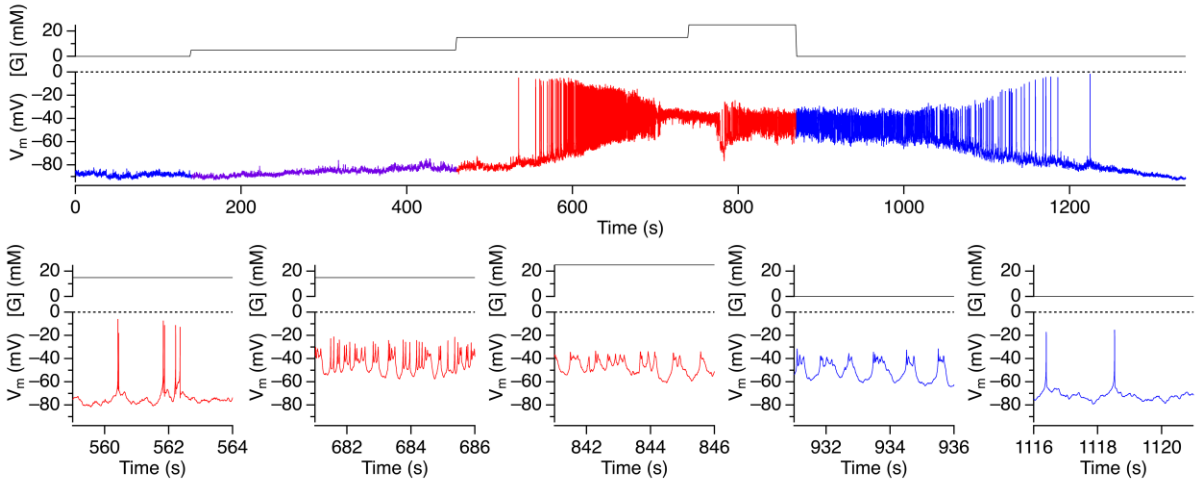
10. β-escin (8 mM in water)
    The final concentration is 8 μM; diluted with the internal solution before each recording session and sonicate

II. **Procedure**

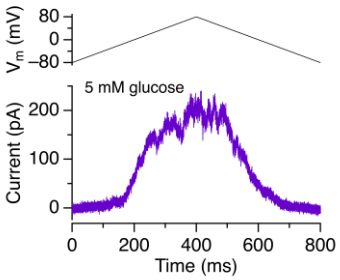
1. Sonicate 8 μM escin diluted with the internal solution in the bath sonicator for >10 min
   4 μM escin is sometimes sufficient
2. Start the perfusion of the recording chamber so that the chamber solution is at 35°C; the perfusion is continuous
3. Take a culture dish with a coverslip with pancreatic 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 (–10mV in size from the holding voltage and 20ms 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-current (I_m) in the voltage-clamp mode; change the external solutions if desired

III. **Data analysis**

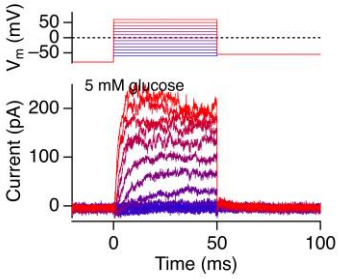
1. Export data to IgorPro (Wavemerics) for data plotting and analysis
2. Data visualization
   a. Current-clamp V_m measurement example (from a cell in an intact islet)



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