INTRACELLULAR CA^{2+} MEASUREMENT WITH WHOLE AND DISPERSED ISLET CELLS

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Date revised: August 18, 2020

I. Equipment Needed

1. Fura-2AM (Life Technologies, Carlsbad, CA)
2. Dual-wavelength fluorescence imaging software (AxioVision- Carl Zeiss Microscopy)
3. Observer.Z1 Microscope fitted with perfusion stage (Carl Zeiss Microscopy)
4. Water-jacketed 5% CO_2 injected 37°C incubator

II. Setup

1. Prepare Krebs buffer (pH 7.4) containing 114 mmol/l NaCl, 5 mmol/l KCl, 24 mmol/l $NaHCO_3$, 1 mmol/l $MgCl_2$ $6H_2O$, 2.2 mmol/l Ca^{2+}, 1 mM Pi, 10 mmol/l HEPES (pH 7.4), 0.25% of BSA (fraction V, fatty acid free)
    a. **Divide solution to 5 bottles:**
        - Buffer A (300 ml): no substrate
        - Buffer B (100 ml): 4 mM amino acid mixture[1] (AAM)
        - Buffer C (100 ml): 4 mM AAM plus 3 mM glucose
        - Buffer D (100 ml): 4 mM AAM plus 16.7 mM glucose
        - Buffer E (100 ml): 0.1 mM IBMX
2. Place all buffers in water bath (37°C) and connect perfusion and oxygen delivery lines
3. Fill perfusion lines
4. Prepare Fura-2AM
    - Dissolve Fura-2AM in 50µl DMSO
    - Add dissolved Fura-2AM to 3ml Krebs buffer supplemented with 5mM Glucose

III. Procedure[2]

1. Load cells with Fura-2AM
    i. Gently wash cells 3x with Krebs buffer
    ii. Load cells with 715µl of Fura-2AM
    iii. Cover dish with foil
    iv. Incubate for 40 min at 37°C
2. Gently wash cells 3x with Krebs buffer

3. Add 1ml of Krebs buffer to the dish
4. Fit dish into perfusion chamber
5. Start perfusion system: flow rate of 1 ml/min
    i. 7 min Buffer A: No Substrate
    ii. 8 min Buffer B: 4mM AAM
    iii. 7 min Buffer C: 4mM AAM plus 3mM Glucose
    iv. 8 min Buffer D: 4mM AAM plus 16.7mM Glucose
    v. 14 min Washout with Buffer A: No Substrate
    vi. 11min Buffer E: 0.1mM KCL
6. Area of analysis is marked and recorded for follow-up immunostaining for cell identification

References

[1] Amino Acid Mixture (AAM) in mM: 0.44 alanine, 0.19 arginine, 0.038 aspartate, 0.094 citrulline, 0.12 glutamate, 0.30 glycine, 0.077 histidine, 0.094 isoleucine, 0.16 leucine, 0.37 lysine, 0.05 methionine, 0.70 ornithine, 0.08 phenylalanine, 0.35 proline, 0.57 serine, 0.27 threonine, 0.073 tryptophan, and 0.20 valine, 2 mM glutamine.

[2] For Dispersed Islets: Region of analysis is determined by a field of view that has easily distinguishable single cell regions such that individual boxes can be drawn around individual cell regions for analysis. For Whole Islets: Region of analysis is determined by a field of view that has an easily distinguishable single whole islet that fits in the analysis view window.