PERIFUSION TO MEASURE INSULIN AND GLUCAGON SECRETION RATE

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I. EQUIPMENT USED FOR EXPERIMENT

1. A computer-controlled, fast-performance LPC system (BioRad Econo pump system with gradient former and buffer selector) that allowed programmable rates of flow and glucose concentration in the perfusate;
2. A water bath (37°C);
3. A fraction collector (Waters Division of Millipore).

II. SET-UP

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 P_i, 10 mmol/l HEPES (pH 7.4), 0.25% of BSA
2. Divide solution to 5 bottles:
    - Buffer A (300 ml): no substrate;
    - Buffer B (200 ml): 4 mM amino acid mixture[1] (AAM);
    - Buffer C (150 ml): 4 mM AAM plus 3 mM glucose;
    - Buffer D (150 ml): 4 mM AAM plus 16.7 mM glucose;
    - Buffer E (150 ml): 4 mM AAM plus 16.7 mM glucose plus 0.1 mM IBMX;
3. Place all bufferes in water bath (37C) and connect perfusion and oxygen delivery lines;
4. Fill perfusion lines.

III. PROCEDURE

1. Using dissecting microscope, hand pick 500-1,000 islets.
2. Place islets on a nylon filter in a plastic perfusion chamber (Millipore, Bedford, MA).
3. Stat perfusion program (flow rate of 1 ml/min) and collect effluent using a fraction collector for insulin and glucagon measurements by radioimmunoassay.

4. Concluding perfusion experiment, nylon filter with embedded islets is collected and frozen at -80 degrees for later determination of DNA and hormone content.

IV. EXPERIMENTAL DESIGN

1. Start perfusion with a 30 min washout period without substrate (Buffer A);
2. Switch to 4mM AAM buffer B for 30 min in the absence of glucose to stimulate glucagon secretion;
3. Switch to Buffer C with 4 mM AAM plus 3 mM glucose for 20 min;
4. Switch to Buffer D with 4 mM AAM plus 16.7 mM glucose for 20 min;
5. Switch to Buffer E with 4 mM AAM plus 16.7 mM glucose plus 0.1 mM IBMX;
6. Switch to Buffer A with no substrate for 20 min;
7. After the initial 20 min of washout, add 30 mM KCl to Buffer A period for an additional 20 min.

V. INSULIN AND GLUCAGON MEASUREMENT

1. Insulin and glucagon concentrations in the outflow samples were measured by the Radioimmunoassay and Biomarkers Core using human insulin RIA HI-14k, EMD Millipore and glucagon RIA GL-32k, EMD Millipore.

Reference

[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.