May 10, 2019

U Mass - Hyperglycemic clamp

DOI
dx.doi.org/10.17504/protocols.io.xzafp2e

Jason Kim[1]

[1]University of Massachusetts

Mouse Metabolic Phenotyping Centers
Tech. support email: **info@mmpc.org**

Lili Liang

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DOI: https://dx.doi.org/10.17504/protocols.io.xzafp2e

External link: https://mmpc.org/shared/document.aspx?id=139&docType=Protocol

Protocol Citation: Jason Kim 2019. U Mass - Hyperglycemic clamp. **protocols.io https://dx.doi.org/10.17504/protocols.io.xzafp2e**

Protocol status: Working
We use this protocol and it's working

Created: February 11, 2019

Last Modified: May 10, 2019

Protocol Integer ID: 20226

Keywords: Hyperglycemic clamp, Islet function, obesity, type 2 diabetes, hyperglycemic clamp measures insulin secretion, hyperglycemic clamp summary, insulin secretion, islet function, abnormal islet function predisposes to the development, abnormal islet function predispose, diabetes, insulin, insulin resistance, cell function

Abstract

Summary:

Hyperglycemic clamp measures insulin secretion and pancreatic β-cell function in awake mice. Islet function is affected by obesity and insulin resistance, and abnormal islet function predisposes to the development of type 2 diabetes.

Materials

MATERIALS

20 % Dextrose injection USP **Pfizer (Hospira)** **Catalog #**NDC0409-7935-19

Note:

Hospira RRID:SCR_003985

Troubleshooting

1 Survival surgery is performed to establish a chronic indwelling catheter at 5~6 days prior to experiment for intravenous infusion. (refer to M1023: Surgery-jugular vein cannulation)

2 Mice are fasted overnight (~15 hours) or for 5 hours prior to the start of experiment.

3 Place a mouse in a rat-size restrainer with its tail tape-tethered at one end.

4 Expose and flush the intravenous catheter using saline solution. Then, connect the catheter to the CMA Microdialysis infusion pump.

5 Collect plasma sample (20 µl) before the start of infusion (basal-0 min) to measure basal glucose and insulin levels.

6 Start infusion of 20% dextrose to quickly reach a target hyperglycemia (~300 mg/dl glucose level) and maintain hyperglycemia by adjusting glucose infusion rates.

7 Collect plasma samples (10 µl each) at 10, 20, 30, 45, 60, 90, and 120 min to measure glucose levels. Adjust glucose infusion rates based on instantaneous glucose levels to maintain at target hyperglycemia.

8 Collect additional plasma samples (10 µl each) at 10, 20, 30, 45, 60, 90, and 120 min to measure insulin concentrations.

9 At the end of experiment, mice are euthanized, and pancreas may be collected for further studies.

10 For data analysis, plasma insulin concentrations may be plotted during the 120-min hyperglycemic clamp experiment, and area-under-curve may be calculated. Area-undercurve of insulin levels during hyperglycemic clamps may be directly correlated with insulin secretion and pancreatic β-cell function assuming there are no effects on insulin clearance rates.

11 Additional plasma samples may be collected to measure serum c-peptide concentrations which may further reflect glucose-induced insulin secretion and pancreatic β-cell function in awake mice.