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Vandy - Hyperglycemic clamp

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We use this protocol and it's working

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Abstract

Summary:

Mice with catheters implanted in the jugular vein (infusions) and carotid artery (sampling) are used for this procedure. The hyperglycemic clamp is used to assess insulin secretory capacity in conscious mice in response to hyperglycemia. Plasma insulin and C-Pedtide concentrations are measured at various times during the 2-hr clamp period.

Materials

MATERIALS

- Infusion Pumps **Harvard Apparatus** **Catalog #**PY8 70-2208
- Stand **Fisher Scientific** **Catalog #**14-670A
- Dual channel swivel **Instech Laboratories, Inc.** **Catalog #**375/D/22QM
- 3- and 4-way stainless steel connectors **Ziggy's Tubes and Wires** **Catalog #**HSCY-25 or HSC425
- Microrenathane tubing (0.033″ OD) **Braintree Scientific** **Catalog #**MRE-033
- Glucose meter and strips **ACCU-CHEK aviva**
- Blunt needle with luer hub **Ziggy's Tubes and Wires** **Catalog #**LHN-E011041 25ga x 0.5″
- Wire stainless steel **Ziggy's Tubes and Wires** **Catalog #**W020304V-1
- Clamp extension **Fisher Scientific** **Catalog #**05-769-7Q
- Connector hook **Fisher Scientific** **Catalog #**14-666-18Q
- 50% dextrose

Reagent Preparation:

Reagent 1: Donor Blood

1. Collect ~ 1 ml of blood from donor mouse in 0.5 ml EDTA tubes.
2. Centrifuge blood (1 min at 16,000 g) and save plasma for preparation of insulin (see below).
3. Resuspend red blood cells (RBC) with heparinized saline (10U/mL).
4. Centrifuge (1 min at 16,000 g), discard supernatant, and resuspend RBC with an equal volume of heparinized saline. Transfer resuspended RBC (donor blood) to a 1.5 ml tube

Note:
Fisher Scientific, RRID:SCR_008452

Troubleshooting

1 Surgical catheterization of the carotid artery and jugular vein in mice at least 5 days prior to the day of the study (refer to protocol for Surgical Catheterization of the Carotid Artery and Jugular Vein).

2 Weigh mouse and start fast (suggested starting time between 7:00 and 8:00 AM) by placing mouse in a plastic container with fresh bedding.

3 Mouse is hooked up to the swivel 4 hours into fasting and basal blood/plasma samples are collected 15 and 5 mins before the start of the clamp (refer to protocol for Hyperinsulinemic-Euglycemic Clamp for detailed set-up and connections).

4 After a total of 5 hr fast, variable infusion of 50% glucose starts. Arterial glucose is increased and maintained at 250-300 mg/dL.

5 Donor blood is infused to jugular vein catheter throughout the study to prevent a fall of hematocrit.

6 Plasma insulin and C-Peptide concentrations are measured at times as described in the following study sheet.

7 At the end of the study, mouse is anesthetized and tissues of interest are harvested and frozen in liquid nitrogen.

TIME (min)	SAMPLE (µl)	GLUCOSE (mg/dl)	Time of infusion change	Glucose infusion rate		HCT	comments
				(µl/min)	mg/kg/min		
-60	Place mouse in tub for acclimation						
-15	50 (G,I)						
-5	100(G,I,C)					*	Start donor blood
0	Variable Glucose Infusion (Clamp to ~300 mg/dl) Donor Blood :7ul/min						
5	50 (G,I)						
10	50 (G,I)						
15	100 (G,I,C)						
20	50 (G,I)						
30	5 (G)						
40	50 (G,I)						
50	5 (G)						
60	50 (G,I)						
70	5 (G)						
80	50 (G,I)						
90	5 (G)						
100	100 (G,I,C)						
110	5 (G)					*	
120	100 (G,I,C)						

I: sample for plasma insulin concentration (25 µl plasma)
C: sample for C-peptide at t = -5,15,100, and 120 (50 µl blood)