Hyperinsulinemic-Hypoglycemic clamp

Version: 1
Replaced by version: N/A
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Summary:

Mice with catheters implanted in the jugular vein (infusions) and carotid artery (sampling) are used for this procedure (V3002). The hyperinsulinemic hypoglycemic clamp involves a constant rate insulin infusion with a fall in blood glucose that is controlled by feed back from regular glucose measurements. Blood glucose is then clamped at a hypoglycemic level. The hypoglycemic clamp is used to test hypoglycemic counterregulation and the functionality of the hypothalamic-pituitary-adrenal axis.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Infusion Pumps	Harvard Apparatus	PY8 70-2208
Stand	Fisher Scientific	14-670A
Dual channel swivel	Instech Solomon	375/D/22QM
3- and 4-way stainless steel connectors	Ziggy's Tubes and Wires	HSCY-25 or HSC4-25
Microrenathane tubing (0.033" OD)	Braintree Scientific	MRE-033
Glucose meter and strips	ACCU-CHEK aviva	
Blunt needle with luer hub	Ziggy's Tubes and Wires	LHN-E011041 25ga x 0.5"
Wire, stainless steel	Ziggy's Tubes and Wires	W020304V-1
Clamp, extension	Fisher Scientific	05-769-7Q
Connector, hook	Fisher Scientific	14-666-18Q
20% dextrose		

Protocol:

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 3 hours into fasting (refer to protocol for Hyperinsulinemic-Euglycemic Clamp for detailed set-up and connections).

4. After a total of 5 hr fast, a constant infusion of insulin starts and glucose is monitored every 10 min and is allowed to fall and is fixed at a hypoglycemic level (~60mg/dL) by adjusting the infusion of 20% glucose.

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

6. Catecholamines and glucagon levels are measured at 0, 30, and 120 min. Insulin is measured at 0 and 120 min.

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

Time (min)	**Sample (ul)**	**Glucose (mg/dl)**	**Time of Infusion Change**	**Glucose Infusion Rate**		**HCT**	**Comments**
				(uL/min)	**(mg/kg/min)**		
0	200 (G,I,N,C,S)					*	Donor RBC
10	10 (G)						
15	10 (G)						
20	10 (G)						
30	160 (G,N,C,S)						
40	10 (G)						
50	10 (G)						
60	10 (G)						
70	10 (G)						
80	10 (G)						
90	10 (G)					*	
100	10 (G)						
110	10 (G)						
120	200 (G,I,N,C,S)						

G: Sample for plasma glucose concentration ~ taken every 10 minutes
I: Sample for plasma insulin concentration (25 µl plasma) ~ taken 0, 120 minutes
N: Sample for plasma glucagon concentration (25 µl plasma) ~ taken 0, 30, 120 minutes
C: Sample of blood for catecholamine (100 µl whole blood) ~ taken 0, 30, 120 minutes

Reagent Preparation:

Reagent 1

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