

Glucose Production Assay in Primary Mouse Hepatocytes

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[Abstract] Hepatic glucose production is a primary determinant of fasting hyperglycemia in type 2 diabetic patients. Glucagon-cAMP-PKA pathway increases, but insulin-PI3 kinase-Akt pathway suppresses glucose production. This assay aims to evaluate the ability of isolated mouse hepatocytes to release newly synthesized glucose mainly from lactate and pyruvate as the substrates (*i.e.* gluconeogenesis) under basal, cAMP-, or cAMP plus insulin-treated condition.

Materials and Reagents

1. Primary mouse hepatocytes
2. Medium199 (Life Technologies, Invitrogen™, catalog number: 11150-059)
3. Fetal bovine serum (FBS)
4. bicinchoninic acid (BCA)
5. Penicillin-Streptomycin, Liquid (Life Technologies, Invitrogen™, catalog number: 15140-122)
6. PBS^{+/+} (Sigma-Aldrich, catalog number: D8662)
7. Dulbecco's modified eagle's medium (DMEM), without glucose, L-glutamine, phenol red, sodium pyruvate and sodium bicarbonate, powder (Sigma-Aldrich, catalog number: D5030)
8. Sodium bicarbonate (Sigma-Aldrich, catalog number: S5761)
9. Sodium L-lactate (Sigma-Aldrich, catalog number: L7022)
10. Sodium pyruvate (Life Technologies, Invitrogen™, catalog number: 11360-070)
11. 100 mM MEM sodium pyruvate solution (100x), liquid (Life Technologies, Invitrogen™, catalog number: 11360-070)
12. 200 mM L-Glutamine (100x), liquid (Life Technologies, Invitrogen™, catalog number: 25030-081)
13. 1 M HEPES buffer solution (Life Technologies, Invitrogen™, catalog number: 15630-080)
14. pCPT-cAMP (Sigma-Aldrich, catalog number: C3912)
15. Insulin (Sigma-Aldrich, catalog number: I9278)
16. Autokit glucose (Wako, catalog number: 439-90901)

17. BCA protein assay kit (Thermo Fisher Scientific, catalog number: 23227)
18. HEPES
19. Glucose production buffer (see Recipes)

Equipment

1. iMark Microplate Absorbance Reader (Bio-Rad, catalog number: 168-1135)
2. BD BioCoat™ Collagen I 6-well Plates (BD Biosciences, catalog number: 356400)

Procedure

1. Primary mouse hepatocytes were cultured in BD BioCoat™ Collagen I 6-well plates (1 x 10⁶ cells per well) in Medium199 supplemented with 5% FBS, penicillin (100 units/ml) and streptomycin (100 µg/ml).
2. 6-48 h after plating, serum-starved overnight in 2 ml/well of Medium199 supplemented with penicillin (100 units/ml) and streptomycin (100 µg/ml) without FBS.
3. Wash the cells by 2 ml/well of warm (37 °C) PBS^{+/+} twice.
4. Replace PBS^{+/+} with 1 ml of glucose production buffer consisting of glucose-free DMEM (pH 7.4) without phenol red supplemented with 20 mM sodium lactate, 2 mM sodium pyruvate, 2 mM L-glutamine and 15 mM HEPES.
5. Incubate cells at 37 °C for 6 h with or without 0.1 mM pCPT-cAMP and/or 100 mM insulin, 0.2 ml of medium was collected and the glucose concentration was measured with a colorimetric glucose assay kit.
6. Collect 0.2 ml of medium from each well.
7. Measure the glucose concentration with a colorimetric glucose assay kit following manufacturer's instruction.
8. Normalized the readings to the total protein content determined from the whole-cell lysates by bicinchoninic acid (BCA) protein assay kit following manufacturer's instruction.

Recipes

1. Glucose production buffer (Please note some component come with the DMEM as noted in paretis)

Components (g/L)

L-Arginine-HCl 0.084 (contained in DMEM)

L-Cystine·2HCl 0.0626 (contained in DMEM)

Glycine 0.030 (contained in DMEM)

L-Histidine·HCl·H₂O 0.042 (contained in DMEM)
 L-Isoleucine 0.105 (contained in DMEM)
 L-Leucine 0.105 (contained in DMEM)
 L-Lysine·HCl 0.146 (contained in DMEM)
 L-Methionine 0.03 (contained in DMEM)
 L-Phenylalanine 0.066 (contained in DMEM)
 L-Serine 0.042 (contained in DMEM)
 L-Threonine 0.095 (contained in DMEM)
 L-Tryptophan 0.016 (contained in DMEM)
 L-Tyrosine·2Na·2H₂O 0.10379 (contained in DMEM)
 L-Valine 0.094 (contained in DMEM)
 Choline chloride 0.004 (contained in DMEM)
 Folic acid 0.004 (contained in DMEM)
 Myo-Inositol 0.0072 (contained in DMEM)
 Niacinamide 0.004 (contained in DMEM)
 D-Pantothenic acid (Hemicalcium) 0.004 (contained in DMEM)
 Pyridoxal·HCl 0.004 (contained in DMEM)
 Riboflavin 0.0004 (contained in DMEM)
 Thiamine·HCl 0.004 (contained in DMEM)
 Calcium chloride (anhydrous) 0.2 (contained in DMEM)
 Ferric nitrate·9H₂O 0.0001 (contained in DMEM)
 Magnesium sulfate (Anhydrous) 0.09767 (contained in DMEM)
 Potassium chloride 0.4 (contained in DMEM)
 Sodium chloride 6.4 (contained in DMEM)
 Sodium phosphate Monobasic (Anhydrous) 0.109 (contained in DMEM)
 L-Glutamine 0.584
 Sodium bicarbonate 3.7
 Sodium lactate 2.24
 Sodium pyruvate 0.22
 HEPES 3.575
 Adjust pH to 7.3, filter using a 0.45 µm filter and store at 4 °C.

Acknowledgments

This protocol was adapted from Sakai *et al.* (2012). The development of this protocol was supported by a Grant-in-Aid for Creative Scientific Research (to M.K.) and a Grant-in-Aid for Scientific Research (C) (21591155 to M.M.) from the Ministry of Education, Culture, Sports,

Science and Technology of Japan, a grant from the National Center for Global Health and Medicine (21S116 to M.M.), a grant from Takeda Science Foundation (to M.M.) and a Novo Nordisk Pharma Insulin Award (to M.M.).

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