Extraction of the SecinH3 from mouse liver

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Introduction

Mice fed with the cytohesin inhibitor SecinH3 for two days develop hepatic insulin resistance that can be identified by reduced liver glycogen levels, increased serum insulin and ketone body levels and decreased serum non-esterified fatty acid. To confirm the presence and identity of SecinH3 in mouse liver, we extracted the compound from liver homogenates with chloroform and identified it by LC/MS.

Reagents

ca. 300 mg mouse liver

chloroform

methanol

PBS

Equipment

HPLC/MS: Agilent 1100 Series HPLC with Bruker Daltonics Esquire Series MS

HPLC columns:

1. 10x4.0 mm with Nucleosil RP18 5 µm
2. 125x2.0 mm with MultoHigh RP18 5 µm

Polytron (Kinematica)

SpeedVac

Procedure

1. 300 mg mouse liver each weighed into 15 mL centrifugation tubes and taken up in 1 ml PBS, 2 mL chloroform added
2. mouse liver homogenized by polytron
3. incubated at RT for 30’ under agitation,
4. Centrifuged 5’ at maximum speed
5. Chloroform phase transferred into fresh centrifugation tube
6. Water phase extracted second time on shaker with 1.5 mL chloroform, 20’
7. Centrifuged 5’ at maximum speed
8. Chloroform phases combined and solvent removed on SpeedVac

9. Residue dissolved in 100 µL/(100 mg liver) methanol, centrifuged 5’ at 14.000 rpm and liquid transferred into HPLC glass vial
10. Fractionated via HPLC on Nucleosil RP18 material 5 µm (10x4.0 mm column); gradient: 0-7 min: 70% water, 30% methanol

7-15 min: 40% water, 60% methanol

15-20 min: 100% methanol;

fractions 9-12 min collected

11. solvent of collected fractions removed by SpeedVac and residue taken up in 50 µl methanol
12. fractionated by HPLC, column: MultoHigh RP18 5 µM (125x2.0 mm column); gradient: 0 min: 50% water, 50% methanol

5 min: 50% water, 50% methanol

25 min: 10% water, 90% methanol

25.1 min: 100% methanol

13. SecinH3 elutes at ca. 20 min; analyzed by MS

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Inhibition of cytohesins by SecinH3 leads to hepatic insulin resistance

by Hafner, M. et al.

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