Adipo C Inhibits Acyl-CoA Synthetase and Reduces Lipid Incorporation into Pancreatic β-Cells

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Introduction

Type II Diabetes (T2D) is a metabolic disease characterized by the body’s inability to use its own insulin to regulate blood glucose levels. It is strongly associated with obesity. Prediabetes is associated with hyperinsulinemia and can lead to insulin resistance and T2D. This state is often induced by exposure to excess nutrients (glucose and fatty acid (FA)). Our lab has previously shown that β-cells exposed to excess nutrients exhibit glucolipotoxicity, or hypersensitivity to glucose that results in hyperinsulinemia of insulin at inappropriately low levels of glucose.

Excess nutrients also cause lipid accumulation within β-cells, which impairs β-cell function. Intracellular activation of FA to its CoA ester by acyl-CoA synthetase (ACS) is required for further FA metabolism, including incorporation into intracellular lipids. As such, elevated ACS expression is linked to harmful lipid accumulation in β-cells. Further, ACS Long Chain Family Member 5 (ACSL-5) is of particular interest as its expression has previously shown that β-cells exposed to excess nutrients (glucose and FA) exhibit glucolipotoxicity, or hypersensitivity to glucose

Methods

Glucose-Stimulated Insulin Secretion (GSIS)
1. Cloud pancreatic INS-1 cells (11 mM) cultured in 11 mM glucose were incubated in 2 mM glucose RPMI medium for 2 hours.
2. Cells were incubated in 11 mM glucose Krebs-Ringer bicarbonate buffer for 3 minutes.
3. Cells were incubated in test solutions of Krebs-Ringer bicarbonate buffer with 1 mM, 2 mM, 3 mM, 4 mM, 6 mM, or 8 mM glucose for 2 hours.
4. INS-1 cells were grown at low (4 mM) glucose and high (11 mM) glucose.

Thin-Layer Chromatography (TLC)
1. INS-1 cells were incubated in 2 mM glucose RPMI medium for 2 hours.
2. Cells were incubated in Krebs-Ringer bicarbonate buffer with or without Adipo C for 30 minutes.
3. Cells were then incubated in Krebs-Ringer bicarbonate buffer and fluorescent bodipy fatty acid (BFA) for 2 hours with or without Adipo C (10 mM), in low (1 mM) or high (12 mM) glucose.
4. Cells were extracted in methanol and collected with a cell scraper.
5. Intracellular lipids were extracted in chloroform:methanol:PBS (2:1:0.75).
6. The reaction was stopped by the addition of Dole’s reagent (500 µM inhibitor).
7. The plates were imaged using a Fuji LAS4000 gel imager (GE) with an excitation wavelength of 488 nm and emission of 515 nm.

ACS Assay
1. INS-1 cells cultured in 4 and 11 mM glucose were extracted in an EBX buffer.
2. Six tri reaction solutions were made for each glucose solution. One (1) had neither inhibitor nor extract, one (1x) had 15 µM extract and no inhibitor, one (2x) had 10 µM extract and no inhibitor, one (3x) had 15 µL extract and 10 µM inhibitor, one (M) had 15 µL extract and 25 µM inhibitor, and one (H) had 15 µL extract and 100 µM inhibitor.
3. Extracts were preincubated with or without inhibitor on ice for one hour.
4. Fluorescent bodipy fatty acid was added to start the reactions; extracts were allowed to react for 20 minutes.
5. The reaction was stopped by the addition of Dole’s reagent andassay product. Four washes were done.
6. Samples from each condition were plated and the fluorescent acyl-CoA product was measured in a TECAN M1000 Per plate reader with excitation wavelength 488 nm and emission of 515 nm.

Research Objective:
To examine the effects of a putative Long-chain Acyl-CoA synthetase 5 (ACSL-5) inhibitor on pancreatic β-cells cultured at both low (4 mM) and high (11 mM) glucose.

Discussion/Conclusions

Glucose-Stimulated Insulin Secretion

Figure 1a. INS-1 cells cultured at 11 mM glucose used for this series of experiments secreted more insulin at basal levels of glucose than β-cells if cultured at lower glucose.

Figure 1b. INS-1 cells cultured at 11 mM glucose used for this series of experiments secreted more insulin at basal levels of glucose than β-cells if cultured at lower glucose.

Figure 2. Glucose-stimulated insulin secretion from INS-1 cells cultured at high (11 mM) glucose. There is a left shift in cells cultured at high (11 mM) glucose compared to cells cultured at low (4 mM) glucose. Although not tested, a typical GSIS pattern of cells cultured at 4 mM glucose is indicated by the red line. An increase in insulin secretion is seen even at 2 mM glucose, indicating insulin hypersecretion

Figure 3. Thin-layer chromatography of lipids from cells incubated fluorescent bodipy fatty acid, with or without Adipo C (10 mM), at high or low glucose. Adipo C blunts the incorporation of the fluorescent-labeled fatty acid into neutral lipids of the β-cell.

Figure 4a. Percent enzyme activity from 4 mM glucose cells activity remaining after Adipo C applied.

Figure 4b. Percent enzyme activity from 11 mM glucose cells activity remaining after Adipo C applied.

Adipo C inhibits ACS activity in INS-1 cells cultured at low (Fig. 4a) and high (Fig. 4b) glucose.

References

Future Research
1. We hope to determine if Adipo C inhibits only ACS isoforms or also inhibits the second enzyme in the pathway for lipid incorporation, acyl-CoA thioesterase.
2. We hope to investigate the different expression levels of ACS isoforms in cells cultured at 4 and 11 mM glucose medium.
3. We want to determine the specificity of Adipo C in the inhibition of ACS isoforms.

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