

系統編號	RC9206-0003		
• 計畫中文名稱	過度糖化最終產物對胰島素訊息傳遞的影響(I)		
• 計畫英文名稱	Effects of Advanced Glycosylation End Products on Insulin-induced Signal Transduciton Pathway (I)		
• 主管機關	行政院國家科學委員會	• 計畫編號	NSC91-2314-B038-025
• 執行機構	台北醫學院醫事技術系		
• 本期期間	9108 ~ 9207		
• 報告頁數	5 頁	• 使用語言	英文
• 研究人員	李宏謨 Lee, Horng-Mo		
• 中文關鍵字	高度糖化終產物；胰島素；訊息傳遞；葡萄糖運送；.epsilon.-羧基甲離胺酸；糖尿病		
• 英文關鍵字	Advanced glycosylation end products (AGEs)；Insulin；Signal transduction；Glucose transport；.epsilon.-Carboxyl-methyllysine (CML)；Diabetes mellitus		
• 中文摘要	<p>過度糖化最終產物(Advanced Glycosylation End Products, 簡稱為 AGEs)。已被證實可影響細胞內的訊息傳遞並且是糖尿病併發症的主要病因。因為第二型糖尿病常見於 AGEs 開始堆積的中老年人,我們假設 AGEs 可以影響胰島素所刺激的 PI 3-kinase 之訊息傳遞路徑,進而影響葡萄糖的運送並形成第二型糖尿病。本研究以分化之 3T3-L1 脂肪細胞,研究 AGEs 對胰島素所引發的 PI 3-kinase 之訊息傳遞路徑和葡萄糖運送的影響。以 BSA-AGEs(300μg/ml)處理分化過之 3T3-L1 脂肪細胞 24 小時後,可發現胰島素所刺激的³H-2-去氧葡萄糖之運送受到抑制。以 AGEs 處理 3T3-L1 脂肪細胞二小時後 IRS-1 出現 Doublet,同時大幅誘導 IRS-2 的蛋白表現。由於 AGEs 亦可增加 PPAR-γ 的表現,而 PPAR-γ 的活化亦可以增加 IRS-2 的蛋白表現,我們推測 IRS-2 的 Upregulation 可能導因於 AGEs 誘導 PPAR-γ 的表現所致。因為 AGEs 構造複雜,為了進一步了解 AGEs 對 PI 3-kinase 相關的訊息傳遞路徑的影響,我們製備了 ε-carboxyl-methyllysine (CML),並證實 CML 亦可抑制胰島素所刺激的³H-2-去氧葡萄糖之運送約 40%。以 CML 處理 3T3-L1 細胞二十四小時後,可以發現 Akt/PKB 的磷酸化和 Akt/PKB 的酵素活性均有明顯的減低。因為對胰島素敏感的葡萄糖輸送者(Insulin-sensitive glucose transporter), GLUT-4,即在 Akt/PKB 的下游;很可能 AGEs 是透過抑制 Akt/PKB 的活性而影響 Glucose transporter 的表現及轉位,並進一步影響³H-2-去氧葡萄糖之運送。為了進一步了解 Akt/PKB 在 AGEs 之抑制作用中所扮演的角色,我們製備了過度表現 Akt/PKB 及 Kinase dead 的 Akt/PKB 突變基因,並且已成功的送入細胞中,證實 Akt/PKB 的蛋白表現和磷酸化情形都能依照預期的增減。我們正進一步研究過度表現 Akt/PKB 的 3T3-L1 細胞是否能逆轉 AGEs 的作用。我們目前的結論是 AGEs 可以透過調節 IRS-1 及 IRS-2</p>		

的訊息傳遞來影響 PI 3-kinase 的訊息傳遞，進而影響³H-2-去氧葡萄糖之運送，過度表現 Akt/PKB 及 Kinase dead 的 Akt/PKB 細胞株將有助於了解其詳細的作用機轉。

Formation of advanced glycation end products (AGEs) is considered a potential link between hyperglycemia and chronic diabetic complications, including disturbances in cell signaling. It was hypothesized that AGEs alter insulin-mediated signaling by interfering with PI 3-kinase dependent pathway. Therefore, we studied the effects of AGEs on the insulin-stimulated PI 3-kinase dependent signaling transduction pathway in differentiated NIH-3T3 L1 adipocytes. Treatment of 3T3-L1 adipocytes with AGEs inhibited the insulin-stimulated ³H] 2-deoxyglucose uptake in a time- and dose-dependent manner. Inhibition of glucose disposal by AGEs is associated with appearance of IRS-1 doublets presumably due to increased Ser³⁰⁷/ phosphorylation and an increased induction of IRS-2 expression. Incubation of 3T3-L1 adipocytes with AGEs increased PPAR- γ . protein expression suggesting AGEs-induced PPAR- γ . expression may affect IRS-2 expression. Furthermore, incubation of 3T3-L1 adipocytes with AGEs reduced Akt/PKB phosphorylation and activation. Given the insulin-sensitive glucose transporter, GLUT-4, is a down stream effector of Akt/PKB, it is possible that AGEs impair the insulin- stimulated glucose transport by inhibiting Akt/PKB activation. In order to demonstrate that Akt/PKB is involved, we have constructed the Akt/PKB overexpression and dominant negative mutant. Transfection of the wild type Akt or overexpression mutants effectively increased the Akt/PKB protein expression and phosphorylation, whereas introduction of the kinase dead mutant inhibits the insulin-induced Akt/PKB phosphorylation. These mutants will prove useful in delineate the mechanism for impaired cellular signal transduction in situations that are associated with diabetes. In conclusion, our data revealed that IRS-1 and IRS-2 undergo differential regulation in 3T3-L1 adipocytes by AGEs. Further works using Akt/PKB overexpression and dominant negative cells will be required to delineate the underneath mechanisms.

- 英文摘要