胰島素調控粒線體中丙酮酸去氫酶機制之探討

Mechanism by which insulin regulates pyruvate dehydrogenase activity in mitochondria

中文摘要

胰島素是人體中主要的同化激素，可以促進肌肉、脂肪組織、及肝臟中的蛋白質和脂質合成，並促進肝醣合成。經由胰島素受器傳導的訊息傳遞路徑主要有二，爲 phosphatidylinositol 3 kinase（PI-3K）/ PDK-1/ Akt 傳遞路徑，以及 MAP kinase 傳遞路徑，然而關於胰島素調控粒線體中丙酮酸脫氫酶複合體的機制，目前則尚未釐清。本實驗室先前研究發現，粒線體中肝醣合成激酶 3b 會與丙酮酸脫氫酶複合體的 E3 次單元體形成蛋白質複合體。在本篇研究中發現，以胰島素刺激 HepG2 細胞，Akt 受到磷酸化而活化，並轉位到粒線體內，進而磷酸化肝醣合成激酶 3b 的第 9 個絲氨酸，抑制其活性，此時丙酮酸脫氫酶的酵素活性上升；在使用 doxorubicin 刺激肝醣合成激酶 3b 的活性增加後，則丙酮酸脫氫酶的活性下降；然而若施予肝醣合成激酶 3b 的抑制劑 TDZD-8 於細胞中，則可以回復肝醣合成激酶 3b 的影響，使丙酮酸脫氫酶的活性受胰島素刺激而上升。此外利用二維電泳和西方墨點法，發現在 doxorubicin 刺激增加肝醣合成激酶 3b 的活性下，丙酮酸脫氫酶的 E2 或是 E3 binding-protein 磷酸化增加，而 TDZD-8 抑制肝醣合成激酶 3b 活性後，則會降低此磷酸化現象。綜和以上研究結果可以推論，胰島素刺激 Akt 磷酸化並轉位到粒線體內，進一步磷酸化肝醣合成激酶 3b 並抑制其活性，使丙酮酸脫氫酶的磷酸化減少，進而調控並增加丙酮酸脫氫酶的酵素活性。這些發現提供了線索，以進一步了解胰島素藉由肝醣合成激酶 3b 調控丙酮酸脫氫酶活性的訊息傳遞路徑。

英文摘要

Insulin is a major anabolic hormone that stimulates synthesis of protein, lipid and glycogen in liver, adipose tissue and muscles. Two main signal transduction pathways downstream of insulin receptor are the phosphatidylinositol 3 kinase/ PDK-1/ Akt pathway and the MAP kinase pathway. How insulin might regulate pyruvate dehydrogenase (PDH) activity in mitochondria is not completely known. Our laboratory has previously found that mitochondrial GSK3b was associated with PDH E3 subunit as a complex. In the present study, we demonstrated that?nAkt was translocated to mitochondria upon insulin stimulation, and the mitochondrial Akt was in its phosphorylated and active state. Activation of Akt is known to phosphorylate and inhibit its downstream enzyme, GSK3b?nat Ser9. Inhibitory phosphorylation of GSK3b maintains PDH at its non-phosphorylated and active state. Consistently, treatment of Hep G2 cells with insulin increased phosphorylation of mitochondrial

GSK-3b, which was associated with an increase of PDH activity. Activation of GSK3b by doxorubicin suppressed the PDH activity, and this effect was reversed by pretreatment of cells with TDZD-8, a GSK3b-specific inhibitor?|?n Furthermore, treatment of Hep G2 cells with doxorubicin increased phosphorylation of PDH E3 binding-protein as revealed by 2D-immunoblotting, and the inhibition of GSK3b?n?nby TDZD-8 abolished this phosphorylation. Taken together, our results suggest that translocation of Akt to mitochondria and subsequent GSK3b?nphosphorylation may regulate PDH activity in mitochondria by phosphorylating the PDH E2 or E3 binding-protein?|?n These findings might provide clues to understand the mechanism by which insulin regulates mitochondrial pyruvate dehydrogenase activity through GSK3b?|