Okadaic acid 誘發大鼠脂肪細胞脂肪分解時細胞內 perilipin 與 beta-actin 的含量變化

Effects of okadaic acid-induced lipolysis on lipid droplet-associated perilipin and beta-actin in rat adipocytes

中文摘要

已有研究證實 okadaic acid 處理脂肪細胞，可以誘發 hormone-sensitive lipase (HSL)的分佈位置改變，由平滑內質網轉移至脂肪滴表面，進而促進脂質分解。但是其對於其他重要的脂肪滴表面蛋白質(如 perilipins 與 beta-actin)的影響卻尚未有研究報告。所以本實驗擬探討在 okadaic acid 處理脂肪分解反應發生時，分析 perilipins 及 beta-actin 這兩種重要的脂肪滴表面蛋白質的含量變化，並探討可能的作用機轉。本實驗取材自大白鼠的脂肪組織，分離出脂肪細胞，加入藥物 isoproterenol 以及 okadaic acid 處理，並以測量甘油的釋放量作爲評估脂質分解的效果。結果顯示 isoproterenol 的正反應對照組以及 okadaic acid 的實驗組，隨著藥物處理的時間逐漸增加，甘油的釋放量都逐漸上升。兩種藥物同時處理時，甘油的釋放量則有顯著的加成作用，顯示兩種藥物可能經由不同訊息傳遞途徑引發脂質分解。藥物處理後分離出脂肪細胞內的脂肪滴，並且利用電泳(SDS-PAGE)與免疫轉漬方法分析脂肪滴表面蛋白質中的 perilipins 以及 beta-actin 的含量，結果發現以 isoproterenol 或是 okadaic acid 處理皆會導致脂肪滴表面的 perilipins 含量下降；但是脂肪滴表面的 beta-actin 含量卻無明顯改變。此外，利用 PKA 的抑制劑(KT 5720), PKC 的抑制劑(calphostin C)與 PKG 的抑制劑(KT 5823)，皆無法有效抑制 okadaic acid 所引發的脂解作用；然而以 vanadate (protein phosphotyrosine phosphatase 的抑制劑)處理卻可以有效地抑制 okadaic acid 誘發的脂解反應。推測 okadaic acid 可能不是經由已知的 PKA, PKC, PKG 等途徑，而是經由抑制 protein phosphatase type 1 (PP1)與 type 2A (PP2A)的途徑，分別活化 perilipins 與 HSL，進而誘發 perilipins 脫離脂肪滴表面，同時促進 HSL 轉移至脂肪滴表面加速脂質分解。然而 vanadate 抑制 okadaic acid 誘發脂質分解的機轉仍待研究深入探討。

關鍵字：脂肪細胞, beta-actin, 脂肪分解, okadaic acid, perilipin

英文摘要

Previous studies have reported that okadaic acid stimulated lipolysis by improving translocation of hormone-sensitive lipase (HSL) from endoplasmic reticulum to surface of intracellular lipid droplets. However, the effects of okadaic acid on lipid

droplet-associated proteins (perilipins and beta-actin) remain unknown. The purpose of this study is to investigate the changes of perilipins and beta-actin after stimulation of okadaic acid. A adipocytes were isolated from rat epididymal fat pads and treated with isoproterenol and/or okadaic acid. Lipolysis was estimated by the measurement of glycerol release. Both isoproterenol (as a positive control) and okadaic acid were found to stimulate lipolysis via a time-dependent manner. The augment of lipolysis was observed in the presence of combination of the two drugs. Therefore, we suggested isoproterenol and okadaic acid might induce lipolysis by different pathways. After drug treatment, intracellular lipid droplets were purified and then lipid droplet-associated perilipins and beta-actin were analyzed by SDS-PAGE and Western blot. Perilipins were decreased in response to isoproterenol or okadaic acid, but beta-actin was no significantly changed neither stimulated or unstimulated cells. In addition, administration of KT 5720 (a PKA inhibitor), calphostin C (a PKC inhibitor) or KT 5823 (a PKG inhibitor) cannot effectively inhibit okadaic acid-induced lipolysis. However, vanadate (a protein phosphotyrosine phosphatase inhibitor) significantly inhibited okadaic acid-mediated lipolysis. Our data suggested that okadaic acid might activate perilipins and HSL by inhibition of type 1 and type 2A protein phosphatases, respectively, but not by activation of PKA, PKC, and PKG. Activated perilipins detach from lipid droplets and activated HSL translocate to lipid droplets, and then accelerate lipolysis. However, the mechanism of inhibition of vanadate on okadaic acid-induced lipolysis still needs to be clarified.

Key words: adipocytes; beta-actin; lipolysis; okadaic acid; perilipin