# YC-1 誘導大鼠脂肪細胞進行脂肪分解之機制研究

## The Mechanisms of YC-1 Induced Lipolysis in Rat Adipocytes

### 中文摘要

YC-1 (3-(5’-hydroxymethyl-2’-furyl)-1-benzylindazole) 是一種化學合成物，可以活化 guanylylcyclase (GC) 並增加細胞內 cGMP 與 cAMP 的濃度。脂肪細胞是體內能量儲存與調控內分泌的重要細胞之一。根據已往的研究指出在促進脂肪細胞進行脂肪分解的途徑中，荷爾蒙先透過與 adrenoceptor 的結合活化 adenylate cyclase (AC) 使細胞內 cAMP 濃度上升，並經由 cAMP-dependent protein kinase (PKA) 途徑磷酸化 hormone-sensitive lipase (HSL) 與 perilipin 來達到誘導脂肪分解的作用。可是近年來發現除 cAMP 之外，cGMP 也對於脂肪分解以及其他與脂肪分解有關之蛋白質也有影響，但其機制並不十分清楚。因此我們以 YC-1 (guanylylcyclase activator) 作爲增加脂肪細胞內 cGMP 含量之刺激物質，來探討 YC-1 與脂肪分解之相關機制。本研究以分離出的大鼠初級脂肪細胞爲研究對象，經 YC-1 處理 2 小時後分別測定游離脂肪酸與甘油之釋放量，以探討 YC-1 是否與脂肪分解有關。另外透過兩種抑制劑：KT5823 (PKG inhibitor) 與 KT5720 (PKA inhibitor) 來研究 YC-1 誘導脂肪分解之途徑，是否爲增加 cGMP 而活化 PKG 來調控，還是間接透過其他途徑增加 cAMP 而活化 PKA，因而造成脂肪分解。接著再以 ODQ (guanylylcyclase inhibitor)處理，抑制 YC-1 與 guanylylcyclase 結合而引起的作用，同時並以 EIA 測定大鼠脂肪細胞內 cGMP 與 cAMP 的濃度，藉此來驗證 YC-1 與脂肪分解的關聯性。

我們的研究結果發現，以 60 　M YC-1 刺激 2 小時有顯著促進脂肪分解的效果,並且由抑制劑的處理證實 YC-1 誘導脂肪分解是經由 PKA 途徑而不透過 PKG 途徑。此外，進一步抑制 guanylylcyclase 後發現可以抑制 YC-1 促進脂肪分解的能力,故在脂肪細胞中 YC-1 是透過 guanylylcyclase 來作用。接著由 EIA 測定發現 YC-1 可以增加細胞內 cGMP 與 cAMP 的濃度，而且 cGMP 的濃度與顯著上升的時間點皆高於且優先於 cAMP；另以 ODQ 抑制 guanylylcyclase 則會明顯抑制 cGMP 的產生。另外加入 insulin 活化 PDE3B 後，發現可以抑制由 YC-1 所促進的脂肪分解現象。最後透過 western blotting 的方式,證實 YC-1 誘導脂肪分解的途徑與 ERK 無關,僅與 cAMP-PKA 途徑有關。由我們的結果證實 YC-1 與脂肪分解有關，且透過活化 guanylylcyclase 後可以藉由增加的 cGMP 來抑制 PDE3B 而減少 cAMP 的水解，間接導致脂肪細胞內 cAMP 濃度的增加,進而活化 PKA 而產生脂肪分解的作用。所以本實驗透過 YC-1 的處理，驗證了在大鼠脂肪細胞中 cGMP 與脂肪分解的關係，並希望以此 cGMP 途徑可以提供研究脂肪分解的另一種新的方向。

## 英文摘要

YC-1 (3-(5'-hydroxymethyl-2'-furyl)-1-benzylindazole), a synthetic chemical compound, has been identified as a direct activator of guanylate cyclase (GC), and can increase intracellular cGMP and cAMP level. Adipocyte is one of the important cells to store energy (in the form of triacylglycerol) and regulate endocrine release. Previous studies have demonstrated that -adrenoceptor of adipocytes are responsible to lipolysis by activating adenylate cyclase (AC) and elevating intracellular cAMP content. The rate-limiting hormone-sensitive lipase (HSL) and perilipin are phosphorylated and activated by PKA. Recently, elevation of intracellular cGMP levels is found to be involved in lipolytic process of adipocyte, but the mechanism is not clear. We used YC-1 (an activator of guanylate cyclase) to increase cGMP concentration, and determined whether YC-1 could improve lipolytic reaction in isolated rat adipocyte. Free fatty acid and glycerol were quantified by spectrophotometry after YC-1 was administrated. In addition, we used PKG inhibitor (KT5823) and PKA inhibitor (KT5720) to examine the mechanism of YC-1 induced lipolysis. Pretreatment with ODQ, a guanylyl cyclase inhibitor, determined whether YC-1 could affect guanylyl cyclase on adipocyte directly.

In this study, we demonstrated that YC-1 (60 M) treated for 2 hr could induce lipolysis in rat adipocyte. The lipolytic reaction was PKA dependent pathway. Furthermore, we found that ODQ could decrease lipolysis and intracellular cGMP, cAMP content. YC-1 induced lipolysis in rat adipocytes through an ERK-independent mechanism by western blotting analysis. Based on the above observation, we suggested that YC-1 induced lipolysis in rat adipocytes through cAMP-PKA dependent pathway not PKG or ERK pathway. We hope this study may provide much information about the regulation mechanisms of cGMP in adipocytes.