ATP 對人類子宮內膜基質細胞中 MAPK 之影響

The Effect of Adenosine Triphosphate on Mitogen-Activated Protein Kinases in Human Endometrial Stromal Cells

中文摘要

腺嘌呤核甘三磷酸 (ATP) 據研究顯示，它會從血小板或自主神經末稍分泌出去。細胞外的腺嘌呤核甘三磷酸 (ATP) 會與細胞膜上的嘌呤受體 (purinoceptor) 結合，進而引發一連串的細胞內訊息傳遞路徑，活化 G-protein, phospholipase C (PLC), diacylglycerol (DAG), protein kinase C (PKC) 之細胞內訊息傳遞路徑。然而對於它的下游訊息傳遞路徑並不清楚。本篇研究的設計主要探討在人類子宮內膜基質細胞，ATP 活化 mitogen-activated protein kinase (MAPK) 的訊息傳遞路徑以及在生理功能上所扮演的角色。西方墨點分析法 (Western blot analysis)；以 ERK1/ERK2 (p42mapk 和 p44mapk) 個別磷酸化形式的單株抗體來做偵測，證實 ATP 活化 MAPK 會隨濃度及時間的不同而產生變化。細胞以 suramin (P2-嘌呤受體拮抗劑)，neomycin (PLC 抑制劑)，staurosporin (PKC 抑制劑) or PD98059 (MEK, MAPK/ERK kinase, 抑制劑) 處理後，其因 ATP 而活化之 MAPK 的表現很明顯被弱化。相對的，P38 和 JNK 並沒有因 ATP 而產生明顯變化。因此證明 ATP 經由 P2-嘌呤受體將訊息傳入細胞內，進而活化 ERK1/ERK2 的訊息傳遞路徑。爲進一步探討 ATP 在細胞所引起早期即時表現的基因變化，細胞以 10uM ATP 處理後萃取其 mRNA，使用經由 mitogen 的訊息傳遞鏈所引起早期即時表現之 23 個基因組膜片，相較於未經 10uM ATP 處理之對照組，證實 early growth response 1 的表現量有明顯增加。本研究所得到的結論，在人類子宮內膜基質細胞，細胞外的 ATP 經由活化 mitogen-activated protein kinase (MAPK) 的訊息傳遞路徑，使得早期即時表現基因 early growth response 1 被表現出來。

英文摘要

ATP has been shown to activate the phospholipase C (PLC)/ diacylglycerol/ protein kinase C (PKC) pathway. However, little is known about the downstream signaling events. The present study was designed to examine the effect of ATP on activation of mitogen-activated protein kinase (MAPK) signaling pathway and its physiological role in human endometrial stromal cells (hESCs). Western blot analysis, using a monoclonal antibody which detected the phosphorylated forms of ERK1/ERK2 (p42mapk and p44mapk, respectively), demonstrated that ATP activated MAPK in a dose- and time-dependent manner. Treatment of the cells with suramin (a P2-purinoceptor antagonist), neomycin (a PLC inhibitor), staurosporin (a PKC

inhibitor) or PD 98059 (a MEK, MAPK/ERK kinase, inhibitor) significantly attenuated the ATP-induced activation of MAPK. In contrast, P38 and JNK were not significantly affected. To study the gene(s) induced by exogenous ATP, mRNA was extracted from hESCs in the presence or absence of 10μM ATP. Gene array for 23 genes associated with members of the mitogenic pathway cascade and immediate early genes revealed that only the expression of early growth response 1 was increased. In conclusion, the effect of ATP on the activation of PKC through P2 receptor acts in concert with ERK and PLC/PKC pathways to induce the expression of early growth response 1 gene in hESCs.