行政院國家科學委員會專題研究計畫成果報告

Thrombin與PGE受體活化MAP kinase機轉之探討

計畫編號: NSC88-2314-B-041-003
執行期限:1998/8/1 - 1999/7/31

主持人: 吳烘　　嘉南藥理學院藥學科

1. Abstract

Extracellular signal-regulated kinase (ERK) is a group of proline-directed kinases regulated by growth factors and G protein coupled receptor agonists and thought to play an important role in proliferation and differentiation. Previous experiments in human erythroleukemia (HEL) cells have demonstrated the occurrence and activation of ERK by PGE_2 and thrombin in a manner dependent on G proteins and protein kinase C. Further exploration of ERK activation in this megakaryocytic cell line has shown that effects of PGE_2 and thrombin were inhibited after treating cells with BAPTA, a calcium chelator, and U0126, a MAP kinase kinase inhibitor. Treatment of cells with genistein, a tyrosine kinase inhibitor, resulted in reduction of ERK activation by PGE2 but not that of thrombin. A role of PI-3 kinase was also indicated by the data that wortmannin significantly inhibits ERK stimulation. Taken together, these data suggest that ERK could be regulated by multiple pathways and PGE_2 mediates its effect via mechanisms dependent on tyrosine kinases and Ca^{2+}. In contrast, protein kinase C, PI-3 kinase, and Ca^{2+} seem to play a more important role in linking thrombin receptor to stimulation of ERK.

本研究計劃發現在HEL細胞可經由不同之路徑調節ERK之活性。實驗結果顯示BAPTA，wortmannin與U0126可阻斷ERK之活化。利用genistein亦發現其可降低PGE2之作用，但卻對thrombin之作用較無影響。這些結果顯示PGE主要經由tyrosine kinases與Ca^{2+}活化ERK，而thrombin受體則需要protein kinase C，PI-3 kinase和Ca^{2+}之參與。

2. Background and Specific Aims

Effects of PGEs in human erythroleukemia (HEL) cells have been characterized previously and have shown that E-series of prostaglandins is coupled to a variety of signaling pathways, such as phospholipase D, adenylyl cyclase, and calcium mobilization (1, 2). Recently, extracellular signal-regulated kinase (ERK) has been demonstrated in these cells and its activity could be stimulated by a variety of agents such ass PGEs, thrombin, PMA, ionomycin, and serum. To better understand the mechanisms that may play a role in cellular functions mediated by these activator, the specific aim of this project is to further explore the potential mechanisms accounted for ERK activation, focusing on the role of Ca^{2+}, tyrosine kinases, PI-3 kinase, and MAP kinase kinase.

3. Results and Discussion

Since PGE and thrombin receptors are coupled to calcium mobilization and probably hydrolysis of phosphoinositide in HEL cells (1,2), first series of experiments was designed to determine whether the calcium chelator, BAPTA, would affect ERK activation. As shown in Fig. 1, BAPTA markedly inhibited PGE2- and thrombin-induced ERK activation, while it had little effect on that of PMA. These results suggest an important role of $[Ca^{2+}]_i$ in effects of thrombin and PGE2.

Secondly, genistein was utilized to test an involvement of tyrosine kinases in ERK activation in HEL cells. Fig. 2 shows that genistein markedly inhibited PGE2-induced ERK activation. In contrast, effect of

thrombin was insensitive to the inhibitor, indicating that thrombin receptor exerts its effect through a tyrosine kinase-independent mechanism. More experiments are needed to elucidate which type of tyrosine kinases is responsible for stimulation of ERK by PGE2.

Thirdly, the role of PI-3 kinase was examined by wortmannin as recent observations have indicated that this kinase is an upstream regulator of ERK signaling pathway (3, 4). Treatment with wortmannin resulted in partial inhibition of PGE2- and thrombin-induced ERK activation (Fig. 3), reflecting an involvement PI-3 kinase in regulation of ERK activity in HEL cells.

Lastly, the possibility that ERK activity is modulated by MAP kinase kinase was tested. In this regard, HEL cells were stimulated after incubating with U0126, a kinase inhibitor with specificity on ERK but not JNK or p38 MAP kinase (5). As expected, neither PGE2 nor thrombin would activate ERK in the presence of U0126 (Fig. 4).

In summary, these results indicate that 1) ERK activity in HEL cells can be regulated by multiple mechanisms; 2) PGE receptor mainly mediates its effect through $[Ca^{2+}]_i$ and tyrosine kinases ; and 3) thrombin's effect is dependent on protein kinase C, PI-3 kinase and $[Ca^{2+}]_i$.

4.. References

(1) Wu, H., Turner, J. T., & Halenda, S. P. (1991) J. Pharmacol. Exp. Ther. 258, 607-612

(2) Wu, H., James-Kracke, M. R., and Halenda, S. P. (1992) Biochemistry 31, 3370-3377

(3) Lopez-Illasaca, M. (1998) Biochem. Pharmacol. 56, 269

(4) Widmann, C., Gibson, S, Jarpe, M. B., and Johnson, G. L. (1998) Physiol. Rev. 79, 143

(5) Favata, M. F. et al (1998) J. Biol. Chem. 273, 18623

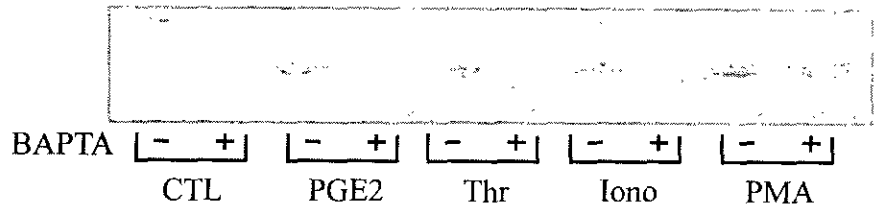

Fig. 1 Effect of BAPTA on ERK activation. HEL cells were incubated with BAPTA/AM for 30 min and then treated for 5 min with various agents. After lysis of cells, the cell lysates were were analyzed by SDS-PAGE and western blotting. A polyclonal antibody specific for tyrosine phosphorylated form of ERK was utilized to detect stimulation of ERK. CTL, control; Thr, thrombin; Iono, ionomycin.

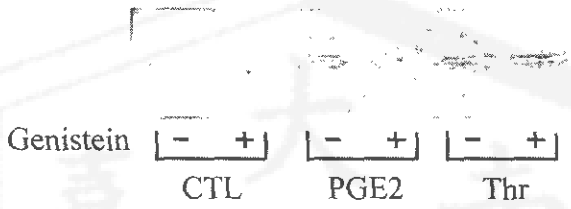

Fig. 2 Effect of genistein on ERK activation. After treatment with or without genistein for 30 min, cells were stimulated by PGE2 or thrombin (Thr) and lysed by sample buffer. ERK activation was then assayed by polyclonal phospho-specific antibody.

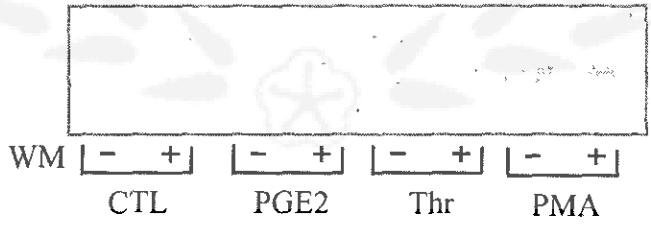

Fig. 3 Effect of wortmannin on ERK activation. Cells were incubated with or without wortmannin for 30 min and stimulated for 5 min. Assay of ERK activity was performed as described in Fig.1.

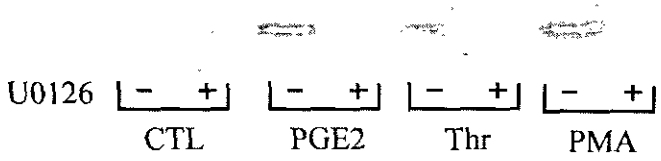

Fig. 4 Inhibition of ERK activation by U0126. Cells were treated with or without U0126 for 15 min aand then incubated with various agents for 5 min. Assay of ERK activity was performed as described in Fig.1.