## cAMP 依賴性的蛋白激酶;對過度糖化最終產物誘導 RAW264.7 吞噬細胞 iNOS 表現和 NO 產生的調控

# Involvement of cAMP Dependent Protein Kinase in BSA-AGEs-induced iNOS Expression and Nitric Oxide Production in RAW 264.7

中文摘要

過度糖化最終產物(AGEs)是糖與蛋白質經非酵素反應及長時間修飾後的產物，它會影響結構和功能改變，並與高齡及長期糖尿病人常見的併發症，如退行性神經變化及糖尿病之腎及血管併發症有關。以前我們曾証明 AGEs 誘導 C6 Glioma cells 之 iNOS 表現可經由 p38 MAPK 路徑。本實驗主要探討 cAMP 依賴性蛋白激酶對於 AGEs 誘導 RAW 264.7 吞噬細胞 (RAW 264.7 細胞) iNOS 表現和 NO 產生的調控作用。AGEs 誘導 RAW 264.7 細胞之 iNOS 表現和 NO 的產生隨著時間與劑量而增加，這種由 AGEs 誘導 RAW 264.7 細胞產生 NO 和 iNOS 的表現可被蛋白激酶 A (PKA)的抑制劑 KT 5720 (1 M) 和 H8 (10 M) 所抑制。PKA 直接活化物 dibutyryl cAMP (Bt2 cAMP) 於不同濃度刺激 RAW 264.7 細胞，結果隨著濃度增加而提升 NO 的產生和 iNOS 的表現。AGEs 刺激細胞內 cAMP 的產生是隨著時間增長而持續增加。為了証明 AGEs 誘導 NO 的產生和 iNOS 的表現不是因 LPS 的污染，本實驗以不同濃度之 LPS 抑制劑 polymyxin B 加入含 AGEs 之 RAW 264.7 細胞，結果發現 AGEs 誘導 NO 的產生不會被 polymyxin B 所影響，證實 AGEs 的作用與 LPS 不同。 AGEs 可於 RAW 264.7 細胞活化 p38 MAPK 的產生，此效果可被 KT 5720 (1 M)，H8 (10 M) 和 SB 203580 (10 M) 所抑制。因此由實驗推測 AGEs 可造成 cAMP 增加，轉而活化 PKA，結果使 p38 MAPK 活化最後造成 iNOS 表現與 NO 的產生。

英文摘要

Advanced glycosylation end products (AGEs) have been implicated in the structural and functional alterations of proteins that occur during aging and long-term diabetes. Previously, we have demonstrated that p38 MAPK is involved in the AGEs-induced iNOS expression in C6 glioma cells. In the present study, roles of cAMP dependent protein kinase in AGEs-induced iNOS expression in RAW 264.7 macrophages were investigated. AGEs caused a dose- and time-dependent increase of nitric oxide (NO) accumulation and iNOS expression in murine RAW 264.7 macrophages. The AGEs-simulated NO production and iNOS expression was dose-dependently inhibited by the PKA inhibitor, KT 5720 (1 M) and H8 (10 M). Consistently, treatment of RAW264.7 macrophages with dibutyryl cAMP results in concentration-dependent

NO release and iNOS induction. On the other hand, AGEs-stimulated cAMP production in RAW 264.7 cells was seen in 1 h and persisted for at least 24 h. The NO production was not affected by polymyxin B, a lipopolysaccharide (LPS) inhibitor, suggesting the NO production is not due to LPS contamination in the BSA-AGEs (AGEs) preparation. AGEs actived p38 MAP kinase in RAW 264.7 cells and this effect was blocked by KT 5720 (1 M), H8 (10 M) and SB 203580 (10 M). The p38 MAPK inhibitor (SB 203580) did not inhibit AGEs-induced cAMP accumulation. In conclusion, our data suggest that AGEs may increase intracellular cAMP, which in turn activates PKA and results in p38 MAPK activation, iNOS induction and NO production in RAW 264.7 macrophages.