一氧化碳誘導環氧化?乙表現之分子機制探討

Study of the molecular mechanisms of carbon monoxide on the induction of cyclooxygenase-2 in macrophage and microglia

中文摘要

血紅素氧化? (heme oxygenase) 是將血紅素 (heme) 降解產生膽綠素/膽紅素、亞鐵離子和一氧化碳 (carbon monoxide)。血紅素氧化?的許多生理功能都與其副產物一氧化碳有關，在許多細胞中，如其具有抗氧化、血管舒張、影響週邊或中樞神經系統的神經傳導活性、抗發炎、抗細胞凋亡或者抗細胞增殖等功能。一氧化碳釋出分子 (carbon monoxide-releasing molecules) 是一類新興藥物試劑，在生物系統中，釋出的一氧化碳可以去調節細胞功能。本篇的研究中，在微小膠質細胞 (microglia) 和巨噬細胞 (macrophage) 中，利用一氧化碳釋出分子 (CO-RMs) 和一氧化碳氣體 (CO gas) (500ppm) 去檢測環氧化?-2 (cyclooxygenase-2, COX-2) 的表現。從西方墨點法 (Western blot) 和反轉錄?-聚合?連鎖反應 (reverse transcriptase-polymerase chain reaction, RT-PCR) 實驗證實，在有脂多醣 (Lipopolysaccharide, LPS)刺激的微小膠質細胞(BV2 and EOC13.31)和巨噬細胞 (RAW264.7) 中，CO-RMs 和 CO gas 可以抑制一氧化氮合成?(inducible nitric oxide synthase, iNOS) 蛋白質和 mRNA 的表現。然而，細胞無論是否受到 LPS 的刺激，CO-RMs 和 CO gas 都會增加 COX-2 的表現。在訊息傳遞方面，CO-RMs 會誘導 MAPKs 及 Akt 磷酸化，且隨著時間的增加磷酸化的表現也就愈明顯。另外，加入了 PKG、p38、Erk、JNK 抑制劑後，會抑制掉 COX-2 的表現。根據實驗結果得知，CO 誘導 COX-2 的表現可能是經由 PKG、p38、Erk 和 JNK 的路徑來傳達。我們更進一步想要探討，在初級腦皮質細胞中經由 CO 處理而誘導 COX-2 表現是否是促成神經細胞死亡的原因。

英文摘要

The enzyme heme oxygenase (HO) degrades heme to produce biliverdin/bilirubin, ferrous iron and carbon monoxide (CO). Many biological functions of HO have been attributed to its enzymatic byproduct carbon monoxide (CO) that exhibits anti-oxidative, vasodilation, and neurotransmission activities in the central or peripheral nervous system, as well as anti-inflammatory, anti-apoptotic, or anti-proliferative potential in many cells. Carbon monoxide-releasing molecules (CO-RMs) are emerging as a new class of pharmacological agents that regulates important cellular function by liberating CO in biological system. In this study, we used both CO-RMs and CO gas to examine the regulation of cyclooxygenase-2 (COX-2) expression in microglia/macrophage. Western blot and RT-PCR analysis demonstrated that CO-RMs and CO gas (500 ppm) significantly inhibited the protein

and mRNA expression of inducible nitric oxide synthase (iNOS) in lipopolysaccharide (LPS)-activated (BV2 and EOC13.31) microglia and (RAW264.7) macrophage. However, CO-RMs and CO gas up-regulated COX-2 expression in the cells with or without LPS. CO-RMs time-dependently induced the phosphorylation of MAPKs and Akt. In addition, the induction of COX-2 could be reversed by PKG, p38, Erk and JNK inhibitors. The results suggest that the induction of COX-2 expression by CO might mediate PKG, p38, Erk and JNK. Further works are to investigate whether induction of COX-2 contribute to the neuron death in primary cortical cells exposed to CO.