生長相關蛋白 GAP-43 Ser41 突變株影響發育期大腦皮質神經元中 Gephyrin 及 GABAA 受器交互作用之探討

The Effect of GAP-43 Ser41 Mutants on the Gephyrin- GABAA Receptor Interaction in Developing Cortical Neurons

中文摘要

神經生長相關蛋白(Growth-associated protein-43; GAP-43)是一種高度表現在發育時期神經細胞軸突生長錐的膜蛋白，GAP-43 負責神經發育及修復時突觸的生長、分支，改變前突觸膜(presynaptic membrane)構造以利釋放神經傳導物。本實驗室先前在蛋白質體研究中利用 MALDI-TOF 發現 GAP-43 會與一受器聚集蛋白(receptor clustering protein) - gephyrin 結合。Gephyrin 的功能是聚集突觸後膜上的氯離子通道神經傳導物受器，包括 GABAA receptor 與 glycine receptor，用以增加抑制型突觸傳遞效能。GABAA receptor 爲大腦中最主要的抑制神經傳導物受器，是由五個次單元組成的氯離子通道，已知 gephyrin 會經由與 GABAA receptor 的 γ2 subunit(GABAARγ2)結合來調控受器的聚集，先前研究發現，投予蛋白質激酶 C (protein kinase C) 抑制劑抑制 GAP-43 活性後， GAP-43 與 gephyrin 的結合有明顯地增加，故推測 GAP-43 的磷酸化可能會改變 gephyrin 與 GABAAR γ 2 的結合，進而影響 GABAA receptor 在後突觸膜上的表現。

在本研究中，我使用初代培養的胚胎期大腦皮質神經細胞(Cortical neurons)爲實驗系統，在發育時期的 cortical neurons 中投予 100 nM PKC inhibitor(Ro318220)處理，發現會減少 GABAAR γ 2 和 gephyrin 的結合，且在細胞膜上的 GABAAR γ 2 表達量減少。爲了模擬不同活性的 GAP-43，我們建立了兩個 GAP-43 S41 位置之突變株(mutant)：將 GAP-43 S41 置換爲 Aspartic acid (S41D)的擬磷酸化 mutant，及 GAP-43 S41 置換爲 Alanine(S41A)的擬去磷酸化 mutant。過度表達 GAP-43S41D 可增加 GABAAR 與 gephyrin 的結合能力，且促進 GABAAR γ 2 在細胞膜上的表現；而過度表現 GAP-43S41A 會減少 GABAAR 與 gephyrin 的結合能力及細胞膜上 GABAAR γ 2 的量。最後，我們發現以離子通道阻斷劑阻斷神經電活性，也會增加 gephyrin 與 GABAAR γ 2 的結合。 因此，本研究證實了神經發育及再生相關生長蛋白 GAP-43 的活性會影響 GABAA receptor 在抑制型突觸形成過程中的表現與分佈，此作用可能是透過 GAP-43 的不同活化態會影響 gephyrin 與 GABAAR γ 2 的結合程度所致。GAP-43 會藉由其 PKC 磷酸化態與 gephyrin 的結合與否，調控 gephyrin 聚合 GABAA receptor 的程度，及 GABAA receptor 在細胞表面的分布，此作用可能進而影響 GABA 突觸的發育與成熟後的傳導效能。

英文摘要

Growth-associated protein 43 (GAP-43) is a membrane protein highly expressed in the growth cone of developing and regenerating neurons. GAP-43 can be activated by protein kinase C (PKC) upon phosphorylation at serine 41 (S41) to promote neurite outgrowth. During neuronal development, GABAA receptors are clustered by gephyrin at γ2 subunit (GABAARγ2) to enhance their synaptic transmission efficacy. In our previous study, we found that blockade of PKC activity by PKC inhibitor RO318220 induce the association between GAP-43 and gephyrin. In the present study, we further found that PKC inhibitor decreased GABAA receptors on cell surface presentation. This evidence suggest that PKC phosphorylation of GAP-43 may regulate the gephyrin-mediated GABAA receptor clustering. To test this hypothesis, we established a wild type GAP-43 expression construct and two GAP-43 mutants by replacing the GAP-43 Ser41, the PKC phosphorylation site with aspartic acid (S41D) or alanine (S41A) to mimic its phosphorylated or dephosphorylated forms, respectively, and transfected these mutants into developing cortical neurons at 4 days in vitro for their overexpression. The results show that the GAP-43S41D-transfected neurons exhibited long and more arborizing neuritis, whereas GAP-43S41A-transfected ones show retracted neurites. In addition, GAP-43S41D overexpression was accompanied by increase in the interaction of GABAARγ2 and gephyrin, GABAARγ2 aggregation in the cell periphery, and the surface expression of GABAAR as revealed by biotinylation assay, whereas GAP-43S41A transfection gave rise to opposite results. Finally, we found that blocked of neuronal activity by voltage-gated sodium channel blocker tetrodotoxin and calcium channel blocker nifedipine also increase the association between gephyrin and GABAARγ2. Together, the results suggest that PKC-mediate GAP-43 phosphorylation promotes the gephyrin-GABAARγ2 interaction and enhances the surface expression of GABAAR. This event depends on neuronal activity, further suggest that the activity-dependent neuroplasticity plays a pivotal role in the establishment of GABAergic synapse and inhibitory neurotransmission.