低鈣狀態對於發育中大白鼠神經系統 BDNF 轉錄之影響

The effect of hypocalcemia on the transcription of BDNF in the developing central nervous system of rat

中文摘要

新生兒低血鈣是臨床上常見的現象，然而新生兒期暫時性低血鈣是否會造成未來神經發展的異常，鮮少有人探討。本實驗室的前趨研究發現，低鈣之培養液會造成大白鼠胚胎神經細胞明顯的死亡，鈣離子濃度越低，細胞死亡的情形越嚴重。表示低血鈣對於不成熟的神經系統可能具有神經毒性。

神經活動依賴之可塑性(activity-dependent neuronal plasticity) 亦即神經細胞的活動會造成本身及鄰近細胞功能及型態的長期性改變是神經細胞發展過程最重要的一環。近來的研究顯示，neuronal plasticity 的形成與神經營養因子(neurotrophin)蛋白質有極密切的關係。其中之一便是 BDNF(Brain-derived neurotrophic factor)。BDNF 的基因含有 8 個 5’ 端不轉譯的 exon(exonI~VIII)及一個 3’ 端的蛋白轉譯 exon (exon IX)。BDNF 的 mRNA transcript 一共有 22 種，exon IX BDNF mRNA 為各 transcript 所共有，也是後來真正轉譯成蛋白質的部份，它的表現會隨著神經細胞的活動而增加，並且這種增加必須伴隨著細胞內鈣離子濃度的上升。Exon I-IX BDNF(BDNF-I) mRNA 則為腦部所特有，在腦外組織表現量極少。過去研究發現，從不同鈣離子通道進入的鈣離子會活化不同的 exon primer，而作出不同的 BDNF mRNA transcript。因此，神經細胞外鈣離子降低有可能改變 BDNF 的表現量。為測試此假設我們使用大白鼠胚胎的大腦皮質細胞，作初級培養，並給予持續低鈣或暫時低鈣處理，觀察神經纖維的生長情形及 total BDNF mRNA(exonIX)以及 BDNF-I mRNA 的表現量是否有所不同並且偵測其神經纖維生長狀況及粗細(活體外實驗)。另外我們也以短期產前皮下注射 calcitonin 導致初生幼鼠有短暫血鈣下降的動物模式，觀察出生後生長狀況，並偵測其海馬迴 total BDNF mRNA 及 BDNF-I mRNA 表現量的變化。細胞模式實驗發現持續低鈣組在 6 天時，輕微低鈣(1.75mM)會使 total BDNF mRNA 下降，但嚴重低鈣(0.96m,0.31mM)反而會上升；BDNF-I mRNA 則會下降。暫時低鈣組在 12 天時的 total BDNF mRNA 及 BDNF-I mRNA 均下降。持續低鈣 0.31mM 組在培養 3 天後神經纖維生長明顯比正常組短；培養到 12 天後，0.96m 及 0.31mM 組的神經纖維直徑明顯較對照組細胞細。另一方面，我們將懷孕母鼠在產前連續二天給予 calcitonin 皮下注射使其產生血鈣降低(活體內實驗)，其所生幼鼠於出生一天時有短暫性低血鈣，且發現低鈣母鼠所生之幼鼠在出生後 7 天內的身長體重明顯低於對照組。此外，低鈣組幼鼠其海馬迴之 total BDNF mRNA 表現量在出生後十四天時有下降的趨勢。

以上結果顯示細胞外鈣離子降低的確會影響 BDNF 轉譯以及神經纖維的生長,但是二者的變化是否有相關則有待未來繼續研究。

英文摘要

Hypocalcaemia is a common problem, yet the long-term sequels of transient hypocalcaemia in developing brain are not known and have not been carefully characterized. Our preliminary study revealed that reduced calcium concentration in the culture medium would lead to rat embryonic neuron death, and the proportion of neuron death is related to calcium concentration. These results suggest that hypocalcemia may be neurotoxic for immature neuron.

"Activity-dependent neuronal plasticity" is an important phenomenon during neuron development. One attractive molecular candidates for modulating synaptic plasticity are the member of neurotrophins (NTs) family, include BDNF(Brain-derived neurotrophic factor). BDNF gene includes eight 5' noncoding exons (exonI~VIII) and one 3' protein coding exon (exon IX).Rat BDNF mRNA contains 22 transcripts, and exon IX mRNA is common region for all transcripts, which could be up-regulated when neuron activity increase. Furthermore, the up-regulation should accompany with increased intracellular calcium concentration. Exon I-IX BDNF(BDNF-I) mRNA transcript is brain-specific. Extracellular calcium could enter into cell via different calcium channel, which has been shown to activate different exon primer and then induces transcription of BDNF mRNA. We proposed that reduction of extracellular calcilum level might alter the expression of BDNF in transcriptional level. To test this hypothesis, we determined the level of total BDNF and BDNF-I mRNA using Real-Time PCR in primary rat cortical neuron cell culture in present of low calcium medium. In addition, we also determined the BDNF transcript in the hippocampus of developing rats born to dam rats received 2-day injection of calcitonin before delivery.

Long-term incubation of cells in low calcium medium induces a transient decrease or increase in total BDNF mRNA and BDNF-1 mRNA, which dependent on the concentration of calcium and incubation period. On the other hand, transient low calcium incubation on Day in vitro 1(DIV 1) induced a delay-onset of reduction of total BDNF and BDNF-I mRNA determined on DV12. Incubation in very low extracellular calcium (0.31mM) medium induced a reduction in the neurite growth and the diameter. Rats born to calcitonin-injected dams rats had low serum calcium level, low body weight gain, and body length growth during the first postnatal week. In addition, total BDNF mRNA level has significant trend of decrease on post-natal day 14.

This result demonstrated that lower extracellular calcium level could affect the transcription of BDNF and neuronal growth. However, whether the reduction of BDNF expression is related to the abnormal neurite growth is required further

investigation.