醣皮質固醇與興奮性胺基酸促進神經再生之協同作用機轉研究

Mechanisms of Glucocorticoid-Excitatory Amino Acid Synergy on Promoting Nerve Regeneration

中文摘要

中樞神經系統損傷後，常伴隨壓力性激素醣皮質固醇(glucocorticoids)及興奮性胺基酸麩胺酸(glutamate)的大量釋放，而造成續發性的神經損傷。然而，臨床上卻發現利用高劑量之合成性醣皮質固醇methylprednisolone (MP)及dexamethasone在治療急性中樞神經系統損傷的病人可產生一定程度之療效。此外，近年亦有報告指出，興奮性胺基酸除了過去所認知的細胞毒性外，可藉由作用在專一性受體進而促成神經細胞之發育及再生。因此，本論文旨在探討醣皮質固醇及興奮性胺基酸對損傷後神經再生的影響及可能之生理及病理作用機轉。

本實驗利用Sprague Dawley (SD)雄性大白鼠背根神經節去軸突(axotomized dorsal root ganglion, AX-DRG)離體培養爲實驗系統，投予齧齒類最主要之醣皮質固醇corticosterone (CORT)及麩胺酸受體之促動劑紅藻胺酸(kainic acid, KA)模擬神經損傷之外在環境，來研究其對神經纖維生長的影響。結果發現早期(離體培養之第一天及第二天)依序投予10 ?峪 CORT及1 mM KA可有效促進神經纖維生長及生長相關蛋白43 (growth-associated protein 43, GAP-43)的表現。若延遲至離體培養之第三天及第四天再投予CORT及KA，非但無法有效增進神經生長，甚至會造成細胞毒性。而此神經滋養作用在阻斷或剔降(knockdown)醣皮質固醇及AMPA/KA受體的活性後便被消除。以上結果顯示，由醣皮質固醇與興奮性胺基酸所模擬的神經損傷環境，在一特定的時間範圍內，會有神經滋養與促進神經再生的作用。

因此，接續之實驗爲探討CORT及KA是如何調控GAP-43的表現與活性。結果顯示，CORT及KA所引發的促神經生長作用可被TrkA 激酶之抑制劑AG-879所阻斷。除了在細胞免疫染色上可發現CORT及KA可增加細胞本體及神經纖維上GAP-43及神經生長因子(nerve growth factor, NGF)的表現外，在西方點墨的實驗中亦證實CORT及KA可明顯地增加TrkA及GAP-43的表現量。由於已知GAP-43須經蛋白激酶C (protein kinase C, PKC)磷酸化後才有促神經生長作用。而PKC除了可磷酸化GAP-43外，亦可在炎性反應相關之調控因子signal transducer and activator of transcription 3 (STAT3)第727胺基酸序列絲胺酸位置(Ser727)進行磷酸化。但此磷酸作用需在STAT3第705胺基酸序列酪胺酸位置(Tyr705)先被Janus kinase (JAK)磷酸化後方可進行。因此，接下來的實驗我們有興趣瞭解的是PKC在CORT及KA所造成之促神經生長作用中所扮演的角色。在投予CORT及KA之前，分別先加入JAK抑制劑AG-490及PKC抑制劑Ro-318220可顯著降低CORT及

KA 之促神經生長作用。在反轉錄-聚合酶鏈式反應(reverse transcription-polymerase chain reaction, RT-PCR)及免疫染色結果中均證實 CORT 及 KA 增加之 GAP-43 之 mRNA 及蛋白質的量在抑制 JAK 及 PKC 的活性後均明顯降低。此外，CORT 及 KA 可促進 GAP-43 及 STAT3 上之磷酸化，而此作用可被 PKC 抑制劑所消減。在活體脊髓損傷之動物模式中，我們採用 MP 取代 CORT，亦可觀察到不論在脊髓或 DRG 中 MP 均可促進 GAP-43 磷酸化的作用。

由於神經生長除了受神經滋養及生長因子所促進外，亦可能受生長抑制性因子所調控。其中，NogoA 爲在髓鞘發現之抑制性蛋白之一，其表現在細胞表面並可與表現在神經元上的專一性受體 Nogo-66 receptor (NgR)結合，而引發下游 RhoA 及 Rho 相關激酶(Rho-associated kinase, ROK)的活化，進而抑制神經纖維的生長。因此，我們同時探討了 CORT 及 KA 是否可能藉由調降神經元及神經膠細胞中 NogoA 的表現及作用而造成促神經生長作用。RT-PCR 及免疫染色結果中發現 CORT 及 KA 可降低 NogoA 之 mRNA 及蛋白質的表現，而此作用在阻斷或剔降 GR 的活性後可被逆轉。CORT 及 KA 合併投予所造成之促神經生長作用，在利用 ROK 抑制劑 Y-27632 取代 CORT 仍然存在。此外，西方點墨法的實驗中我們亦發現單獨投予 CORT 即可顯著降低 RhoA 的表現量。另在星狀膠細胞(astrocyte)之離體培養系統中，亦證實 CORT 可顯著抑制星狀膠細胞中 RhoA 的表現量，而投予 MP 可明顯降低由興奮性毒性所誘發的 NogoA 表現量。

綜合以上實驗結果，我們發現神經損傷初期投予醣皮質固醇及興奮性胺基酸可協同促進神經纖維之生長。此作用機制可能與 CORT 透過作用在 GR 而抑制 NogoA 及 RhoA 的表現有關。此現象可協同促進後續由興奮性胺基酸透過 AMPA/KA 受體所造成之 PKC 活性相依之促神經生長機制，進而達到神經修復的作用。

英文摘要

Elevation of glucocorticoids and excessive glutamate release are the two major stress responses that occur sequentially during traumatic CNS injury which can result in secondary neuronal damage. Nevertheless, the synthetic glucocorticoids methylprednisone (MP) and dexamethasone are frequently used for treating the acute phase of CNS injury. Activation of glutamate receptor (GluR) has also been shown to influence several aspects of neuronal function during developing nervous system and neurite regeneration. These observations imply that glucocorticoids and glutamate might be beneficial for nerve repair. However, the mechanisms of this assumption are still limited.

In this study, we applied corticosterone (CORT), the main adrenal glucocorticoids in rat, and a glutamate receptor agonist, kainic acid (KA), in cultured axotomized rat

dorsal root ganglion neurons (AX-DRG) to mimic an environment of nerve injury to investigate the effects of glucocorticoid and excitatory amino acid on neurite outgrowth. Our results revealed that combined treatment of CORT and KA (CORT+KA) resulted in synergistic enhancement of neurite outgrowth and expression of growth-associated protein 43 (GAP-43) when applied as early as 1 and 2 days in vitro culture (1 and 2 DIV), but not 3 and 4 DIV in a sequential application order. In addition, application of CORT+KA caused neurotoxicity at 3 and 4 DIV but not at 1 and 2 DIV. The neurotrophic effects mediated by CORT+KA were abolished by down-regulation of glucocorticoids receptor (GR), AMPA/KA receptor, and receptor tyrosine kinase A (TrkA) activities.

GAP-43 is known to promote neurite extension when phosphorylated by protein kinase C (PKC). Furthermore, PKC can phosphorylate the signal transducer and activator of transcription 3 (STAT3), a cytokine-related transcription factor, at Ser727, which is phosphorylated primarily by Janus kinase (JAK) at Tyr705. In further study, we examine the role of PKC in this stress-induced growth-promoting effect. In the cultured DRG neurons, the JAK inhibitor AG-490 and the PKC inhibitor Ro-318220 reduced the CORT+KA-enhanced neurite growth when applied prior to CORT and KA treatment, respectively. Both AG-490 and Ro-318220 diminished the CORT+KA-enhanced GAP-43 expression, phosphorylation, and axonal localization. Furthermore, treatment the cell with CORT+KA synergistically phosphorylated STAT3 at Ser727 but not at Tyr705. Similar phenomena were observed in an animal model of acute spinal cord injury (SCI), in which phosphorylation of GAP-43 and phospho-Ser727-STAT3 was elevated in the injured DRG at 4 hr after the impact injury. Further treatment with the therapeutic glucocorticoid methylprednisolone enhanced the phosphorylation of GAP-43 in both the DRG and the spinal cord of SCI rats.

NogoA has been identified as a myelin-associated inhibitory protein that interacts with Nogo-66 receptor (NgR) to activate downstream signaling RhoA as well as Rho-associated kinase (ROK) and plays an important role in limiting axonal growth. In this study we further elucidated the role of NogoA-mediated inhibitory pathway in the synergistic effect of CORT+KA on neurite growth. RT-PCR analysis and immunofluorescent staining revealed that the suppressed NogoA expression by CORT+KA was abolished by transfecting cultured DRG with GR siRNA. Using Y-27632 to block ROK, the downstream signaling of NogoA receptor, showed a similar effect as CORT and KA synergy in promoting neurite growth. Glucocorticoids not only decreased RhoA expression in DRG neurons and astrocyte but also suppressed NogoA expression in reactivated astrocyte.

Taken together, these results suggest that the neurotrophic effects of

glucocorticoids on axonal regeneration might require facilitation of excitatory stimulation at early stage of nerve injury. CORT acting on GR to down-regulate NogoA and RhoA might play a key role in facilitating the subsequent PKC-dependent neurite growth upon activation of AMPA/KA receptors.