1. 利用受體結合實驗分析 Dextromethorphan 及安非他命對大白鼠腦部 N-methyl-D-aspartate (NMDA) 接受器的作用 2. 腹腔內注射成年 C57BL/6J 小鼠 N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)對腦部 Glial Cell Line-derived Neu

1. The Ligand Binding Analysis of the Effects of Dextromethorphan and Amphetamine on the N-methyl-D-aspartate (NMDA) Receptor 2. Regulation of Brain Glial cell Line-Derived Neurotrophic Factor (GDNF) Expression by Intra-peritoneal Injection of N-methyl-4

中文摘要

1．許多與受體結合的離子通道（ligand-gated ion channels），其性質會隨著發育期間而改變。由本實驗室先前研究發現長時問給予母鼠嗎啡所生之幼鼠，在出生後第 14 天其腦部ＮＭＤＡ受體的數目比正常幼鼠較少，推測這些長期暴露於嗎啡環境下的幼鼠，其腦中ＮＭＤＡ受體除數目上的”量”的改變外，是否也包含了”質”的變化？因此我們以Ｄextromethorphan（ＤＭ），一種ＮＭＤＡ受體拮抗劑取代[3H]TCP 的結合，來了解嗎啡組幼鼠大腦皮質ＮＭＤＡ受體內的結合能力是否發生變化?另外我們也利用成年大白鼠大腦皮質比較ＤＭ及其兩種代謝物：Ｄextrophan（ＤＴ）、Ｍethoxymorphinan（ＭＭ）在ＮＭＤＡ離子通道內對於取代[3H]TCP 結合能力的差異。結果顯示嗎啡組幼鼠在不同天數間以ＤＭ取代 [3H]TCP 的結合能力並無顯著不同，推測以ＤＭ這個藥物的角度看來，在此模式下的發育過程中雖ＮＭＤＡ受體有數目上的改變，但有可能透過有別於ＤＭ的結合位置來影響其變化。而在成年大白鼠大腦皮質中ＤＭ、ＤＴ及ＭＭ對取代 [3H]TCP 的結合能力也無明顯變化。

另外在過去本實驗室利用受體結合實驗及測定鈣離子內流實驗，發現安非他命抑制了ＮＭＤＡ所引起的神經生理及病理作用，我們推測安非他命對ＮＭＤＡ受體有直接的抑制作用。因此我們以大白鼠大腦皮質利用受體結合的方法， 想再進一步的探討不同結構的安非他命：methamphetamine（ＭＡ），d-amphetamine（ＤＡ），l-amphetamine（LA）對ＮＭＤＡ受體的抑制作用是否具有差異性？由此實驗證明三種不同結構的安非他命都可以直接抑制ＮＭＤＡ受體的活化，且同樣的具有二處不同的效價作用區，其作用力在高效價區為 LA＝ＤＡ=ＭＡ，低效價區則為 LA＝ＤＡ> ＭＡ，顯示結構上的差異會影響安非他命於ＮＭＤＡ受體上的結合能力。

2．安非他命的使用會導致腦中多巴胺神經細胞的傷害，而ＧＤＮＦ可能是已知可以提高多巴胺神經細胞存活率之各種神經營養因子中，最有效的一種。近年來神

經膠細胞源神經營養因子(glial cell line-derived neurotrophic factor, GDNF)正受到廣泛的注意及重視，它可保護腦中多巴胺神經細胞免於腦部傷害或神經毒素的損傷，並可幫助其再生，因此，GDNF 的研究在臨床神經醫學上有很重要的意義。而 MPTP（N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine）是一種神經毒素，它會選擇性的殺死由黑質體投射到紋狀體的多巴胺神經元，因此我們選用了 14-15 週大的 C57BL/6J 雄性成年小黑鼠，在急性腹腔內注射給予 MPTP（N-methyl-4-phenyl-1,2,3,6-tetrahydro pyridine）20 mg/kg 1 小時，24 小時，3 天及 7 天後將其斷頭犧牲取大腦皮質、紋狀體及小腦後進行 RT-PCR 的生化檢測方法，偵測在此動物模式下腦中神經膠細胞是否可代償性的分泌 GDNF？又，我們更進一步研究 bax，c-fos，c-jun mRNA 的表現來了解由 MPTP 所造成的毒性是否與細胞計劃性凋亡有關？結果顯示 GDNF 的表現在大腦皮質、紋狀體及小腦中皆無明顯的改變，而 c-fos 在打藥後的三個腦區中於 1 小時即出現明顯的表現；bax 則在打藥後 3 天及 7 天的三個腦區中具明顯的表現。證明在 紋狀體中 MPTP 對多巴胺神經細胞造成傷害後，會藉突觸間的連繫使大腦皮質及小腦神經細胞也受到損害，進而使神經細胞走向細胞凋亡的路徑。

英文摘要

1. Dextromethorphan, a non-competitive antagonist of N-methyl-D- aspartate (NMDA) receptor, attenuate NMDA receptor-mediated response by binding to a site in the receptor-coupled ion channel. [3H]N-(1-[2-thienyl]cyclohexyl)-3,4- piperidine（[3H]TCP）, a radiolabelled ligand binding to the phencyclidine binding site in the NMDA receptor-coupled channel, has been widely used to determine the expression of the NMDA receptor in the brain tissues. Our previous study has demonstrated a ontogenic change in the affinity of [3H]TCP binding in developing rat brain. In particularly, combined prenatal and post-natal exposure to morphine induced a time-specific change in the density of the NMDA receptor in cortex and hippocampus. We further asked whether this specific change is a fundamental alteration in the property of the NMDA receptor-coupled channel, including a parallel change in the affinity of DM in binding into this channel. To address this issues we determine the potency of DM in displacing the binding of [3H]TCP in cortical tissues derived from rats with age of 7, 14, 30 and 60 days. Morphine group rats were rats born to dams rats received bi-daily injection of morphine since one week before mating till the end of first month after delivery. Control group rats were rats born to dams rats received saline injection only. Our results showed that no change of affinity for TCP binding and Ki for DM in inhibiting [3H]TCP was found between control and morphine group rats during these examined post-natal days. Furthermore, we determined whether the two major metabolites of DM, namely the dextrorphan (DT) and 3-methoxymorphinol

(MM), may be also potent in inhibiting the [3H]TCP in the NMDA receptor-coupled channel, in the membrane prepared from cortex tissues of adult normal rats. We found DT and MM can concentration dependently inhibiting [3H]TCP binding with IC50 similar to that of DM, but no significant difference between DM, DT and MM in displacing the [3H]TCP binding.

Amphetamine, a psychostimulant, has been found by our previous study to have antagonist effect by directly acting at multiple sites on the NMDA receptor. We further demonstrate the stero-specifity of this antagonizing effect by exam the methamphetamine(MA),d-amphetamine(DA) and l-amphetamine(LA)in displacing [3H]TCP binding to study its stereo-specify. The results suggested that all these drugs could inhibit [3H]TCP binding with two potencies. The rank order of inhibiting affinity of high potency effect is LA=DA=MA, and of low potency effect is LA=DA>MA, suggest that the stereo-specific of the two potency effect of amphetamine is different, and the binding sites for these two effects of amphetamine on the NMDA receptor is not identical.

2. Administration of MPTP（N-methyl-4- phenyl-1,2,3,6- tetrahydropyridine）to mammals causes damage to the dopaminergic pathway and produce symptoms similar to that observed in Parkinson’ s disease. Glial cell line-derived neurotrophic factor（GDNF）has been proposed as a useful therapeutic agent in the treatment of Parkinson’s disease. The gene expression of GDNF was enhanced following various brain insults. So the objective of the present study was to exam the expression of GDNF mRNA following MPTP treatment in C57BL/6J mice. We also investigated alterations in cell death effector gene expression, bax, and the immediate-early genes, c-fos and c-jun, induced by MPTP in C57BL/6J mice cortex, striatum and cerebellum. In conclusion, MPTP-induced dopaminergic neurotoxicity does not elicit any changes in the expression of endogenous GDNF mRNA in the adult mouse cortex, striatum and cerebellum. And we found that MPTP could induce a transient increase in the c-fos expression and induce a late onset increase in the bax gene expression in all brain regions examined. This results indicate that MPTP-induced neurotoxicity involve apoptosis process which could occur in brain regions not related to the dopaminergic system.