(1) 安非他命對 NMDA 在大白鼠大腦皮質神經細胞上所引起 45Ca2+ 蓄積之作用 (2) 在大白鼠大腦皮質神經細胞培養中發生的細胞凋零的特徵

(1) The Effect of Amphetamine on the N-Methyl-D-Aspartate Induced Intracellular 45Ca2+ Accumulation.(2) The Characterization of the Apoptosis in Rat Primary Cortical Cell Culture.

中文摘要

1)安非他命是一種精神刺激劑,有關安非他命的中樞神經作用的研究過去大都著重在與多巴胺神經傳導系統的關係,然而最近有許多證據顯示安非他命所造成的行爲敏感化作用 (behavior sensitization) 能被 N-methyl-D-aspartate receptor (NMDA) 的拮抗劑所阻斷， NMDA 受體的活化參與了安非他命的中樞神經的作用。過去本實驗室利用[3H]TCP 受體結合的方法及 45Ca2+ accumulation 的實驗，觀察安非他命對 NMDA 受體的作用，結果顯示安非他命可以直接抑制 NMDA 受體的活化。在本實驗中我們將再次確認安非他命可以抑制 NMDA 所引起的 45Ca2+ accumulation，並且比較右旋 (d-amphetamine)、左旋安非他命 (l-amphetamine)及甲基安非他命 (Methamphetamine)對 NMDA 所引起的 45Ca++ accumulation 是否具有抑制作用及其個別的強度。

結果顯示，安非他命是 NMDA 的拮抗劑且安非他命對 NMDA 引起的 45Ca2+ accumulation 有兩階段的抑制作用。而不同結構的安非他命對 NMDA 引起的 45Ca2+ accumulation 的抑制作用強度是差不多的。

(2) Apoptosis (細胞凋亡)又稱爲 Programmed cell death (計劃性細胞死亡)，顧名思義，它是由細胞自行調控死亡，是一種選擇性的主動死亡方式。本實驗利用大白鼠大腦皮質神經細胞的培養觀察 apoptosis 的現象，我們發現隨著培養天數的增加，神經細胞的密度漸漸降低而且有許多細胞呈現 apoptosis 的特徵包括神經細胞縮小、核質濃縮、及產生許多 apoptotic bodies 等。因此我們判定這種神經細胞培養有自生性的 apoptosis,根據此種觀察,我們想要利用其它重要方法來確定此種判斷。我們除了利用倒立式相位差顯微鏡直接觀察並照下神經細胞的外型之外,並且以 LDH response 測量細胞毒性,及利用 TUNEL assay 偵測 apoptosis 的細胞，以及利用 RT-PCR 的方法偵測一些 mRNA 的表現量，包括 bax、c-fos、c-jun 等。此外，我們並分別在神經細胞離體外培養第 6、12、18 天時投與 NMDA、MK-801、morphine、naloxone 等藥物觀察它們對神經細胞的影響，瞭解他們是否會促進或抑制神經細胞的 apoptosis。

結果發現大白鼠大腦皮質初代神經細胞培養 (rat primary neuronal cell culture)中有自發性的 apoptosis，可作為一種研究 apoptosis 的模式。而且直接以顯微鏡及以 TUNEL assay 觀察都發現：apoptosis 細胞的數目會隨著培養天數增加而上升。再偵測培養 1 到 22 天的細胞的 mRNA，以 ß-actin 作為 internal control，發現 c-fos mRNA 的量在第 20 天有明顯的上升，但是 NSE、NF-M、bax、c-jun mRNA 在不同天數則沒有明顯差異。在第 6 天的 culture 上不論 NMDA、MK-801、morphine、naloxone 對細胞內 LDH 釋放的量都沒有明顯的作用，細胞型態在不同時間也沒有明顯變化，但是投與 NMDA 與 MK-801 會降低 c-fos mRNA 的量。在第 12、18 的細胞投與 NMDA 與 MK-801 則會降低 NSE 和 NF-M mRNA 的量。在第 12 天、18 天的 culture 上 NMDA 組的 LDH 值比 control 組高，在倒立式相位差顯微鏡下觀察到 NMDA 組細胞外型呈現明顯的 necrosis，到 24 小時細胞已明顯的死亡 (Figure 27,29)，此時以 TUNEL assay 偵測 apoptosis 的細胞，發現 neuron 的數目較少，而且存活下來的細胞也幾乎 100% 發生 apoptosis。可見得 NMDA 會同時造成 neuron 的 necrosis 與 apoptosis。

英文摘要

(1) Recent investigations have indicated that amphetamine, a psychostimulator, produces its central effect by activation of the NMDA (N-methyl-D-aspartate) receptor, a subtype receptor of the glutamate receptor. Our previous study using [3H]TCP receptor ligand binding to assess this receptor/channel complex had shown that amphetamine could directly inhibit the NMDA-coupled ion channel by acting at multiple sites on the receptor. In the present study, we examined this specific action of amphetamine and compare the effect of d-amphetamine、l-amphetamine、Methamphetamine on the NMDA receptor-mediated 45Ca2+ accumulation.
This study confirm that amphetamine could direct inhibit the function of NMDA receptor, and the d-amphetamine、l-amphetamine、Methamphetamine have similar effect on the NMDA receptor-mediated 45Ca2+ accumulation.
(2) Apoptosis (also termed programmed cell death, PCD) is a mode of cell death in which the cell plays an active role in its demise. In the present study, we investigate the characteristic of apoptosis in the primary cortical neuronal culture. We find that the cell in our rat primary cortical cell culture undergoing apoptosis by the examination of microscope. We confirm the presence of apoptosis in this culture by several biochemical methods. Including taking picture of neuronal culture under microscope, examine the apoptotic cells by TUNEL assay and immunocytochemistry, and quantify the mRNA level of bax, c-fos, c-jun by RT-PCR. Besides, we also examine the LDH response of culture cells. In addition, we also detect the effect of NMDA (N-methyl-D-aspartate) (100 M), MK-801 (10 M), morphine (10 M),

and naloxone (10 M) on the apoptosis of this culture in DIV6, 12, 18 culture. We find that our rat primary cortical cell culture undergoing spontaneous apoptosis expressed as increase in the LDH level, increase in the number of TUNEL positive neuron in a two-phase manner. This apoptotic phenomenon is accompanied with increase gene expression of c-fos, and likely the expression of bax gene. Addition of NMDA to culture also induces significant increase in LDH response accompanies with apoptosis. But MK-801, morphine, or naloxone has no effect on LDH response and apoptosis. However, NMDA or MK-801 decrease c-fos on DIV 6 culture, but increase c-fos on DIV 18 culture. Morphine and naloxone didn't have any effect on the c-fos gene expression. NMDA increase apoptotic neurons on DIV 12 and 18 culture, but it has no effect on DIV 6 culture. This study confirm that NMDA increase apoptosis in this culture, but MK-801, morphine, or naloxone didn't have any effect on the apoptosis in this culture.