重覆使用安非他命及檳榔後腦內之神經化學變化

Neurochemical Events Mediated by Repeated Administration of Amphetamine and Areca Nut Extracts

中文摘要

第一部份

檳榔素（Arecoline）是一種檳榔中主要的生物鹼，也是毒蕈膽鹼素受體（muscarinic cholinergic receptor）的部份同類物（partial agonist），最近被證實可以用來增強阿茲海默症（Alzheimer's disease）病人的記憶。爲了解長期投與檳榔素後腦內之神經化學變化，我們以檳榔素每公斤 10 毫克的劑量經腹腔注射投與大白鼠十四天後，取其大腦皮質切片經 [3H] 肌醇（[3H]-inositol）標定後，檢查了毒蕈膽鹼素受體所誘發的磷脂醯肌醇（phosphatidylinositol）水解。我們發現長期投與檳榔素會抑制約 40% 的碳醯膽鹼素所造成的磷脂醯肌醇水解效應。爲了進一步了解其抑制的機轉，本研究接著檢查了檳榔素在大白鼠大腦皮質切片中的直接作用。我們發現檳榔素可以刺激磷脂醯肌醇水解的訊息傳遞路徑，但是其最大效應只有碳醯膽鹼素（Carbachol）的 30%，其 EC50 約爲 30?M。同時加入檳榔素及碳醯膽鹼素所造成的磷脂醯肌醇水解效應減少了 70%，而其 IC50 也約爲 30?M。如果將大白鼠大腦皮質切塊暴露在不同濃度的檳榔素下一小時，然後洗除檳榔素，再檢查碳醯膽鹼素所造成的磷脂醯肌醇水解效應，則仍有劑量依存性的抑制作用，其 IC50 約爲 10?M。此一去敏感作用發生的極快，在 15 分鐘內即有一半的最大抑制作用，而其最大抑制作用則發生在一小時後。此去敏感作用可在洗除檳榔素後，經一小時的時間回復，同時也證實此去敏感作用不是因爲檳榔素造成的 Gq?G11?下調節作用（down regulation）。當我們以大白鼠大腦皮質切片製備成細胞膜後，經由受體鍵結物結合實驗（receptor-ligand binding）發現檳榔素處理一小時後，受體的總數和控制組並無明顯差別；但是如果將大白鼠大腦皮質製成 synaptoneurosomes，細胞外的受體數目（maximal ligand binding；Bmax）明顯因檳榔素的存在而減少，但受體結合力（binding affinity；Kd）卻無顯著的變化，更有趣的是減少的受體數目又可經一小時的溫孵而再生。綜合這些發現，我們的結論是長期投與檳榔素後碳醯膽鹼素所造成的磷脂醯肌醇水解會受到抑制，此抑制作用主要的影響應是檳榔素造成受體的隔離作用（receptor sequestration）所致。

第二部份

注射安非他命（Amphetamine）可能

在行爲上造成長期性的改變，這其中包括了行 爲敏感化，毒品的耐受性和依賴性。而安非他命也可經由調控甲基天門冬酸受體（NMDA receptor），在斜紋體（striatum）和阿控伯核（nucleus accumbens）的中間或腹側部上的神經細胞誘導出立即早期基因（immediate early genes）(c-fos 和 jun B，c-fos 和 jun B 都是轉錄因子（transcriptional factor）並可調控一系列基因的表現。爲了探討腦部訊息核糖核酸（mRNA）經由重覆間歇性處理安非他命後表現的情況，我們取大白鼠給與安非他命每公斤 5 毫克的劑量連續腹腔注射十四天，待大白鼠產生行 爲敏感化之後，取出海馬體（hippocampus）和斜紋體的訊息核糖核酸以便來做差異顯 示法（PCR Differential Display）。經放射性物質標定聚合酵素連鎖反應的產物所產生的片段，可以清楚的和連續十四天注射生理食鹽水的大白鼠所得到的訊息核糖核酸來作比較。我們在海馬體和斜紋體分別找到了 10 個和 3 個有差異的片段，並重覆以差異顯示法 確定這些差異的表現之後，這些片段均經由聚合酵素連鎖反應再放大，並選殖（clone）到 TA 載體（TA vector）中。我們將繼續以北方墨點法（Northern Blot）、全長互補去氧核糖核酸（full length cDNA）的分子選殖（molecular cloning）和定序（sequencing）等實驗，來確定這些片段的特性。

英文摘要

[Part One]

Arecoline, a major alkaloid in Areca nut stimulates muscarinic cholinergic receptors and has been used to improve memory retention in patients with Alzheimer's disease. To understand the effects of arecoline administration on the muscarinic cholinergic signaling pathway, the rats were injected with arecoline (i.p.) at a concentration of 10 mg/Kg or repeatedly injected with the same dose of arecoline for 14 days, and the carbachol-stimulated phosphoinositide breakdown in rat brain cortical slices was examined. In vivo administration of arecoline resulted in a 40% inhibition of carbachol-stimulated phosphoinositide turnover in rat brain cortical slices. In rat brain cortical slices, arecoline was a partial agonist with maximal effects of 30% of the maximum as obtained with carbachol, the EC50 was about 30?M. Co-addition of arecoline and carbachol inhibited the carbachol-stimulated phosphoinositide breakdown by 70%, the IC50

was also about 30?M. Consistent with the in vivo observation, pretreatment of rat brain cortical slices with arecoline in vitro resulted in a dose-dependent inhibition of carbachol-stimulated [3H]-inositol monophosphate accumulation. When arecoline was added 1 hour prior to the reaction following by extensive washes, the IC50 for arecoline was approximately 10?M. The desensitization occurred rapidly with half-maximal inhibition occurring at 15 minutes and maximal inhibition achieved within 60 minutes. The desensitization can be recovered by incubating the slices without arecoline for 1 hour after arecoline pretreatment. The desensitization was not due to Gq?G11?down regulation. When membranes prepared from rat brain slices previously treated with arecoline for 2 hours were used for receptor-ligand binding studies, the receptor numbers and binding affinities were not changed by arecoline treatment. However, when synaptoneurosomes were used for the receptor-ligand binding studies, arecoline pretreatment decrease the maximal ligand binding (Bmax) without inducing any marked change in binding affinity (Kd). The influence can be reversed by incubating synaptoneurosomes in the absence of arecoline for 2 hours. Taken together, these data suggest that the underlying mechanism by which phosphosinositide turnover is inhibited is due to arecoline-induced receptor sequestration.

[Part Two]

Amphetamine administration may result in long term changes in the behaviors including sensitization, tolerance and dependence. Amphtamine also induces immediate early genes, c-fos and jun B in the neurons of the medial and ventral striatum and nucleus accumbens via NMDA receptor. C-fos and jun B are both transcriptional factors that regulate a series of gene expression. In order to screen for brain region specific mRNAs that are expressed after repeated intermittent amphetamine treatment, PCR differential display was employed. In the present studies, the rats were repeatedly injected with amphetamine (i.p.) at a concentration of 5 mg/Kg for 14 days. After the rats have developed behavioral sensitization, mRNAs from hippocampus and striatum were extracted for PCR differential display. Radiolabeled PCR products of banding patterns were compared with the control mRNAs isolated from the rats after 14 days' repeated intermittent saline administration. This appproach identified 10 specific PCR bands from hippocampus and 3 specific PCR bands from striatum were altered by amphetamine administration. Similar PCR differential display reactions were performed to confirm the differential expressions. Following confirmation, these PCR bands were reamplified and then cloned into TA cloning vector. Future works are required to further characterize the PCR products. These works include Northern Blot, molecular cloning and sequencing of the full length cDNA.