Overexpression of Bcl-2 protects differentiated PC12 cells against beta amyloid-induced apoptosis through inhibition of NF-kB and p38 MAP kinase activation

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Activation of the apoptosis program by an increased production of beta-amyloid peptides (Aβ) has been implicated in the neuronal cell death of Alzheimer's disease. Bcl-2 is a well demonstrated anti-apoptotic protein, however, the mechanism of anti-apoptotic action of Bcl-2 in Aβ -induced apoptosis of neuronal cells is not fully understood. In the present study, we therefore have investigated the possibility that the overexpression of bcl-2 may prevent Aβ-induced apoptosis through inhibition of the pro-apoptotic activation of the transcription factor NF-κB and the p38 MAP kinase in differentiated PC 12 cells. Aβ increased apoptosis of differentiated PC12 cells in a dose dependent manner. Treatment of Aβ resulted in increase of caspase-3 activity and activated NF-κB and p38 MAP kinase. Overexpression of Bcl-2 protected against Aβ-induced apoptosis, and suppressed the activation of caspase-3, NF-κB and p38 MAP kinase. Moreover, inhibition of p38 MAP kinase with a specific inhibitor SB 203580 attenuated Aβ-induced apoptosis. This inhibitory effect was correlated well with the inhibition of Bcl-2 expression and NF-κB activation, indicating that p38 MAP kinase serve as a signaling pathway in the Aβ-induced cell death process. These results suggest that Bcl-2 overexpression protects against Aβ-induced cell death of differentiated PC12, and its protective effect may be related to the inhibition of the activation of NF-κB and p38 MAP kinase.

[PB3-4] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

REGULATION OF MUSCARINIC RECEPTOR-MEDIATED sAPP RELEASE BY PLA2-RELATED PATHWAYS.

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Chronic inflammatory processes are associated with pathology of Alzheimer's disease(AD). The expression of both cyclooxygenase-2(COX-2) and phospholipase A2(PLA2) appears to be strongly activated during AD, indicating the importance of inflammatory gene pathways as a response to brain injury. Stimulation of heterotrimeric G protein-coupled receptors including muscarinic receptors activates cytosolic PLA2 and receptor-mediated activation of PLA2 generates free fatty acids (i.e., arachidonic acid). Likewise the Gq protein-coupled receptors including muscarinic (M1, M3) receptors, metabotropic glutamate receptors, and bradykinin receptors have been indicated to regulate release of sAPP which occurs in the A beta domain, prevents A beta deposition. We examined whether the muscarinic receptor mediated sAPP release is distinctly regulated by PLA2 related pathway in SH-SY5Y cells which express endogenous muscarinic M3 receptors. sAPP release into the culture media was analyzed by immunoblotting with mono clonal antibody 22C11. Treatment of cells with a irreversible PLA2 inhibitor, manoalide, blocked the secretion of both basal and oxoM-stimulated sAPP, whereas activated by a PLA2 activator, mellittin. In addition, PLA2 products, the arachidonic acid and PGE2, strongly increased the secretion of both basal and oxoM-mediated sAPP. These results implicate PLA2-related pathway regulates basal APP processing and secretion and is involved in muscarinic receptor-mediated sAPP release in this cells. Next, to investigate whether the down

streams of PLA2 pathways, COX and lipoxygenase(LOX) pathways, are regulate the muscarinic-mediated sAPP release, we examined the effects of COX and LOX inhibitors on sAPP release. The COX and LOX inhibitors partially increased constitutive sAPP release, but failed to show significant change in oxoM -stimulated sAPP release. The COX and LOX inhibitors only reduced arachidonic acid induced sAPP release. Although COX and LOX are maybe involved in constitutive release of sAPP, our results indicated that muscarinic receptor mediated sAPP release is regulated by the generation of arachidonic acid through PLA2 activation rather than COX and LOX activities.

[PB3-5] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

Dihydropyrimidinase related protein-2 expression in focal ischemic rat brain and hypoxia-induced PC12 cell

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Ischemia-induced changes in protein expression may provide important insights into the mechanisms of cellular damage and their potential recovery. In the present study, to investigate protein patterns changed in ischemic condition, the cortical and striatal tissue samples from the permanent and transient ischemic rat brain obtained by middle cerebral occlusion were analysed by proteomic approchese using 2D-PAGE and MALDI-MS. Among proteins shown to clear differences in their expression level, the dihydropyrimidinase related protein-2 (DRP-2) was identified in produce under ischemic condition, and therefore, DRP-2 might be a candidate new molecule that could play an important role in brain ischemic mechanism.
The DRP-2 is known to be equivalent to TOAD-64(TUC-2), CRMP-2 and Ulip-2. Changes of expression level of this protein in ischemic brain were confirmed by western blotting using monoclonal DRP-2 antibody (C4G). The C4G antibody labeled three separated bands. Unexpectedly, the major band (64 kDa) and the upper band (66 kDa) with less density were decreased but the lower band (62 kDa) was clearly increased in their intensity according to the ischemic duration in both cortical and striatal region of the MCA-occluded rat model. Ischemia is commonly known as a condition of glucose and oxygen deprivation. Therefore, we tried to investigate whether the DRP-2 exprssion level also changes in hypoxia- induced PC12 cell, and obtained the similar pattern to that of the MCA-occluded rat model. While the 64 KDa band dcreased faint gradually, the 62 kDa band was increased. Furthermore, these results were observed according to hypoxic duration and media condition which was absence or presence of glucose and serum. It can be postulated that the differentially changed three bands observed in ischemic rat brain and hypoxia-induced PC12 cell by DRP-2 antibody represent distinct isoforms of DRP-2 generated as result of alternative mRNA splicing, proteolysis, or its post-translationally modified DRP-2. The possibility of proteolysis and alternative splicing of DRP-2 mRNA during ischemic condition is being tested.
In conclusion, at least three isoforms exist, and their expression level changed differentially in ischemic condition of rat brain and hypoxia-induced neuronal cells. Although the role of each isoform of this protein in still not known exactly, our results suggest the involvement of DRP-2 in ischemic and hypoxic condition, and these could be provided useful information to elucidate pathophysiological mechanisms and therapeutic strategies of stroke.

[PB3-6] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

Characterization of choline transport in immortalized rat brain capillary endothleial cell lines (TR-BBB)