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Parkinson's disease (PD) is a widespread neurodegenerative disorder. Even though PD has been studied in many aspects, it is still unknown the molecular signaling mechanisms linking reactive oxygen species (ROS) and neuronal apoptosis in PD. A better understanding of cellular mechanisms that occur in Parkinson's disease is essential for development of new therapies. In this study we investigated the signaling molecules involved in neuronal apoptosis induced by 6-hydroxydopamine (6-OHDA) in human SK-N-SH neuroblastoma cells as a model cellular system. Treatment of SK-N-SH cells with 6-OHDA increased nitric oxide generation and apoptosis. N(G)-monomethyl-L-arginine (NMMA), a NOS inhibitor, prevented 6-OHDA-induced cell death. In addition, 6-OHDA also induced time-dependent phosphorylation of extracellular signal-regulated protein kinase (ERK1/2) and cyclic AMP regulatory binding protein (CREB), which was not dependent on phosphatidylinositol 3-kinase(PI3-K). Furthermore, 6-OHDA also increased Bax expression but decreased bcl-2 level. Blocking of ERK1/2 activation with the upstream inhibitor PD98059 prevented 6-OHDA-induced cell death and changes of the ratio between Bax and Bcl-2. These data suggest that ERK1/2 play an important role in 6-OHDA-induced neurotoxicity.

[PB3-2] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

A novel potassium channel opener, KR-31378, protects cortex neurons from oxidative injury by restoring antioxidant enzyme activities and glutathione levels

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Neuronal hyperexcitability followed by high level of intracellular calcium and oxidative stress play critical roles in neuronal cell death in stroke and neurotrauma. Hence, KR-31378, a novel benzopyran derivative was designed as a new therapeutic strategy for neuroprotection possessing both anti-oxidant and potassium channel modulating activities. In the present study, we tested for its neuroprotective efficacy against oxidative stress-induced cell death in primary cortical cultures and further investigated its neuroprotective mechanism.
Incubation of cortical neurons with KR-31378 protected FeSO4-induced apoptotic as well as necrotic cell death in a concentration dependent manner. The protective effect of KR-31378 was neither mimicked by other potassium channel openers nor abolished in the presence of KATP channel blockers, indicating that its effect was not related K+ channel opening activity. The mechanism of protection is rather attributable to the antioxidant property of KR-31378 since it suppressed the intracellular accumulation of ROS and ensuring lipid peroxidation caused by FeSO4. We further studied its effect on antioxidant defense, enzymatic and nonenzymatic system. Prooxidant, FeSO4, resulted in decrease of catalase and glutathione peroxidase activities, which were restored by KR-31378 treatment. In addition, it attenuated the depletion of glutathione contents caused by FeSO4. Taken together, modulation of antioxidant enzyme activities and glutathione metabolism may contribute to the antioxidant property of KR-31378 by which it exerts a beneficial effect in oxidative stress-induced brain injury and it represents a potentially useful therapeutic agent for the ischemic brain injury

[PB3-3] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

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