POSSIBLE NEUROTOXICITY OF LEVODOPA

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Although the cause of Parkinson's disease is still unknown, the oxidative stress has been implicated in the pathogenesis of the disease. This theory postulates that normal metabolic processes in the nigrostriatal dopaminergic system may lead to loss of the neurons, and that iron-dependent membrane lipid peroxidation may play an important role in the neuronal death. In this symposium, several pieces of our recent research concerning iron-dependent lipid peroxidation facilitated by catechols (levodopa and dopamine) are introduced. (1) Levodopa and iron form strong oxidizing complexes and induce lipid peroxidation (LPO) in phospholipid lip` ` ´ Uosomes. Neuronal cell cultures are destroyed by this LPO. Substantia nigra obtained from rats is very vulnerable to this LPO. (2) Synthetic melanin prepared by autooxidation of levodopa promotes LPO in the presence of iron. Effects of scavenging agents indicate that this LPO is mediated by superoxide, but not by other oxygen free radicals.` ` ´ Ç (3) Catechols induce mobilization of ferritin iron. The released iron (i.e., loosely-bound iron) is available to iron-dependent LPO. These data suggest that biochemical and morphological characteristics of substantia nigra, which are concomitant with its functional role, meet the requirement to provoke iron-dependent lipid peroxidation. Inappropriate administ-ration of levodopa may accelerate neuronal death in the nigrostriatal dopaminergic system.

NEURIT-STIMULATING EFFECT OF CORTEXIN AND EPITHALAMIN IN ORGANOTYPIC CULTURE OF NERVE TISSUE

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The effect of the natural brain peptides-cortexin and epithalamin were investigated in 5-day organotypic cultures of dorsal root ganglion (DRG) neurons and explants of brain tissue from 10-11 day old chick embryo. Cortexin and epithalamin were extracted from brain tissue of large horned cattle /molecular weight about 10 kDa/. Cortexin (2-100 ng/ml), epithalamin (200 ng/ml) showed neurite-stimulating effect in DRG cultures as compared to the control explants. The concentration of cortexin 100 ng/ml showed stimulating effect in cortical cultures, and the concentration of epithalamin 200 ng/ml 100 ng/ml showed the stimulating effect in cultures of subcortical structures as compared to the control explants. So cortexin and epithalamin can support structural and functional homeostasis of the cell populations which secreted these peptides. These peptide bioregulators with neurite-stimulating effect can be used in the treatment of neurodegenerative diseases, as has been shown recently for neurotrophic factors.

TRANSCRIPTIONAL RESPONSE TO OXIDATIVE STRESS IN CULTURED NEURONAL AND GLIAL CELLS

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To clarify the effects of oxidative stress in cultured rat mesencephalic cells, we examined cell viability and changes in DNA-binding activities of the transcription factors, using Trypan blue exclusion and electrophoretic mobility shift assay. Neurotoxin 6-OHDA or H2O2 reduced the viability of both types of cells in time and concentration dependent manner, however, cultured glial cells were more resistant to oxidative stress than cultured neuronal cells. Both neurotoxin dose-dependently decreased the DNA-binding activities of AP-1 and cAMP-responsive element binding protein (CREB) in cultured neuronal cells, reflecting a decrease in the number of surviving neuronal cells. In contrast, DNA-binding activities were increased in glial cells. In addition, incubation with 6-OHDA or H2O2 induced a rise in glutathione level in cultured glial cell. These results suggest that the effects of oxidative stress on transcription factors is different in neuronal and glial cells. In glial cells, oxidative stress induced the activation of transcription factors and may regulate the level of antioxidants such as glutathione. This up-regulation of antioxidant in glial cell appears to reflect the glial resistance and the defence mechanisms in the brain against oxidative stress.

TRANSFECTION OF BPV-LAC Z PLASMIDS IN PRIMATE CV1-P AND SH-SY5Y CELL CULTURES

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Bovine papilloma virus (BPV)-plasmids were developed to express foreign genes in the host cells. In general, BPV-plasmids are nontoxic, noninfectious and can be delivered into all types of cells replicating independently on the host cell genome. In this study, the BPV-plasmids expressing bacterial lac Z gene were transfected with cationic liposomes in ape CV1-P fibroblast and human SH-SY5Y neuroblastoma cell lines. The lac Z gene transfections were detected using a chemiluminescence assay for beta galactosidase. Optimal transfection conditions were obtained in the absence of serum using 2.5-10 μg of DNA and 12.5-50 μg Dosper with Dosper/DNA-ratio of 5-10, a transfection time of 6-12 hrs followed by an additional incubation up to 24 hrs. Dosper caused some cell damage especially in neuroblastoma cells. Since the toxic effect was prevented by serum, the neuroblastoma cells were transfected in the presence of serum, although serum itself decreased the transfection efficiency. Thus, BPV plasmids were successfully transfected and expressed in two primate cell lines, suggesting that these constructs can be used to develop a gene therapeutic treatment for the degenerative brain diseases and other diseases.