（第３号様式）

学 位 論 文 要 旨

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論　文　名　正常および疾患マウスの中枢神経系におけるプロサポシンの発現

学位論文要旨

Prosaposin, a 66-kDa glycoprotein, is the precursor of saposins A, B, C, and D, which act as cofactors in the hydrolysis of sphingolipids by lysosomal hydrolases. In lysosomes, PS is proteolytically processed to generate four sphingolipid activator proteins, known as saposins A to D, which are required for hydrolysis of sphingolipids by several lysosomal exohydrolases. Many functions have been attributed to secreted PS, which is reportedly a trophic factor in the nervous and reproductive systems, being present in milk and cerebrospinal and seminal fluids. The PS gene contains 15 exons. It is transcribed into several mRNAs, resulting from alternative splicing of the 9-bp exon 8. In situ hybridization has shown abundant PS expression in the epithelial cells of the choroid plexus and various grey matter areas, including the cortex and hippocampus. Besides its role as the precursor protein of saposins, PS is also a neurotrophic factor capable of inducing neural differentiation and preventing cell death. A neurotrophic sequence has been identified in 14 amino acids located in the N-terminal part of saposin C and has been attributed to PS neurotrophic activity.

In order to research the function of PS in central nerve system, we choose two models of mice, one is Parkinson disease mice, and the other is mdx mice.

Parkinson’s disease (PD) is a progressive neurodegenerative disorder with cardinal clinical features of bradykinesia (slowness), postural instability (imbalance), rigidity, resting tremor, autonomic dysfunction, and psychiatric manifestations. The principal neuropathologic feature of PD is selective degeneration of midbrain dopamine-producing neurons of the substantia nigra (SN) parscompacta (SNpc) that make up the nigrostriatal pathway, which results in depletion of dopamine in the caudate nucleus and putamen (collectively termed the striatum). To explore the mechanisms responsible for the effects of prosaposin on neuronal survival in PD, we treated dopaminergic neurons with PS18, which comprises the hydrophilic sequence of rat saposin C. It was found to resist protease cleavage and cross the blood–brain barrier intact when administered systemically. In the present study, we used 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 1-methyl-4-phenylpyridinium ion (MPP+)-induced dopaminergic neurotoxicity in C57BL/6J mice or SH-SY5Y cells and explored the protective effect and mechanisms of PS18 on dopaminergic neurons. Treatment with 2.0 mg/kg prosaposin-derived 18-mer

peptide (PS18) significantly improved behavioral deficits, enhanced the survival of tyrosine hydroxylase-positive neurons, and decreased the activity of astrocytes in the substantia nigra and striatum in MPTP-induced PD model mice. In vitro, a Cell Counting Kit-8 assay and Hoechst 33258 staining revealed that co-treatment with 300 ng/ml PS18 and 5 mM MPP+ protected MPP+-induced nuclear morphological changes and attenuated cell death induced by MPP+. We also found that PS18-FAM entered the cells, and the amount of time that PS18-FAM was retained in the cytoplasm of MPP+-treated cells was shorter than that of untreated cells. In addition, PS18 showed protection from MPP+/MPTP-induced apoptosis in the SH-SY5Y cells and dopaminergic neurons in the PD model mouse via suppression of the c-Jun N-terminal kinases/c-Jun pathway; upregulation of Bcl-2; downregulation of BAX, attenuating mitochondrial damage; and inhibition of caspase-3.

Duchene muscular dystrophy (DMD) is a fatal genetic disease caused by mutations in the DMD gene, leading to dystrophin deficiency. DMD is caused by a mutation in the X-linked dystrophin gene; it is a recessive genetic disease characterised by alterations in the neuromuscular system, and metabolic and structural disorders of the central nervous system (CNS), which cause mental retardation and metabolic damage. While muscle wasting is prominent, the CNS is also affected in DMD, with non-progressive intellectual and/or cognitive impairment being observed in about one-third of patients with DMD. The dystrophin-deficient mdx mouse is a model of human DMD. Recently, we reported low levels of PS in muscles in mdx mice compared with C57BL/10 mice. The distribution of PS in the brains of juvenile and adult mdx mice was investigated by immunochemistry, Western blotting, and in situ hybridization. Immunochemistry revealed lower levels of PS in the cytoplasm of neurons of the cerebral cortex, hippocampus, cerebellum, and choroid plexus in mdx mice. Western blotting confirmed that PS levels were lower in these brain regions in both juveniles and adults. Even with low PS production in the choroids plexus, there was no significant PS decrease in cerebrospinal fluid (CSF). This discrepancy may be explained by decreased PS intake from the CSF due to decreased expression of PS receptors (GRP37, GRP37L1) in mdx mice. In situ hybridization revealed that the primary form of PS mRNA in both normal and mdx mice was Pro+9, a secretory-type PS, and the hybridization signals for Pro+9 in the above-mentioned brain regions were weaker in mdx mice than in normal mice. We also investigated mitogen-activated protein kinase signalling. Stronger activation of ERK1/2 was observed in mdx mice, ERK1/2 activity was positively correlated with PS activity, and exogenous PS18 stimulated both p-ERK1/2 and PS in SH-SY5Y cells.

These results suggest that prosaposin may play an important role to protection of neurons survival.

キーワード（３～５）	Prosaposin, Parkinson’s disease, prosaposin-derived 18-mer peptide, mdx mice, SH-SY5Y.