Cell Death in Neuromuscular Diseases

Endogenous Neuroprotective Molecules and Their Mechanisms in the Central Nervous System

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Functions of the central nervous system (CNS) are based on a complex neural network. It is believed that the CNS has several neuroprotective mechanisms operated by neurons, glia and other types of cells against various types of neuronal damage. Since mature, differentiated neurons are not able to divide, it is important to protect neurons from damage prior to death. The neuroprotective effects of a number of pharmaceutical agents and natural products against necrosis and apoptosis of the CNS neurons have been reported, thus this review will mainly discuss several endogenous neuroprotectants and their mechanisms.

Key words neuroprotectant; central nervous system; neuronal damage

1. INTRODUCTION

The neurons of the central nervous system (CNS) form a complex network to perform CNS functions such as thinking, memory, and regulating actions. Once the neural network has been damaged by cerebrovascular diseases, neurodegenerative disorders, or nerve injuries, it is difficult to reconstruct the network so that it functions properly like before the damage. Therefore, it is believed that protection of neurons from the impairment prior to death is a reasonable strategy for the treatment of nerve disorders and injuries. Although a large number of publications have discussed neuroprotection by pharmaceutical agents and natural products against oxidative stress, inflammation, excitotoxicity in disorders^{1–4)} and traumatic injuries^{5,6)} of the CNS, we would like to mainly discuss endogenous neuroprotective factors in this review.

2. PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE

Pituitary adenylate cyclase-activating polypeptide (PACAP) was first isolated as a 38 amino acid hypothalamic neuropeptide.⁷⁾ PACAP exerts its function by binding to three different G-protein coupled receptors, PAC₁, vasoactive intestinal polypeptide receptor 1 (VPAC₁) and VPAC₂. Although the PAC₁ receptor selectively binds to PACAP, VPAC₁ and VPAC₂ receptors also respond to vasoactive intestinal peptide with high affinity.⁸⁾ Vasoactive intestinal peptide, which has 68% homology with PACAP, is also known as a neuroprotectant, but its adenylate cyclase stimulating activity is at least 100 times less than that of PACAP.^{7,9)} All three receptors are widely distributed with various levels in different regions of the CNS. PACAP acts as a neurotrophic factor promoting cortical neurogenesis¹⁰⁾ and as a neuroprotectant blocking neuronal death induced by the excitotoxin *N*-methyl-D-aspartate (NMDA) or serum deprivation in cortical neuron cultures.¹¹⁾ Chen *et al.*¹²⁾ revealed that endogenous PACAP deficiency in mice caused increases in infarct volume and neurological

deficits compared with wild type mice after middle cerebral artery occlusion (MCAO), and that subsequent systemic administration of PACAP to PACAP-deficient mice restored lesion volume and the deficits to the same level of amelioration as in wild type mice. In addition, Ohtaki *et al.*¹³⁾ demonstrated that the neuroprotective mechanisms of PACAP against ischemic neuronal death involved the suppression of cytochrome *c* release regulated by bcl-2 and the activation of extracellular signal-regulated kinase (ERK) and signal transducers and activators of transcription (STAT)-3 associated with interleukin (IL)-6. As indicated by a large number of *in vitro* and *in vivo* studies, PACAP is recognized as a general and potent neuroprotectant.^{14–16)}

3. HEPATOCYTE GROWTH FACTOR

Although hepatocyte growth factor (HGF) was identified as a potent mitogenic factor for hepatocytes,^{17,18)} it also regulates the survival and proliferation of other types of cells in lung and kidney. HGF and its receptor, the c-Met tyrosine kinase receptor, play essential roles in embryonic development because HGF- or c-Met-deficient mice show embryonic lethality.^{19,20)} HGF and the c-Met receptor are expressed in the brain and demonstrate a neuroprotective action against the death of neurons *in vitro* and *in vivo*. HGF prevented nuclear translocation of apoptosis inducing factor and then neuronal death induced by NMDA treatment in primary cultured hippocampal neurons.²¹⁾ Continuous administration of HGF into the brain had a marked protective effect on delayed neuronal death in the hippocampus of gerbils after transient ischemia.²²⁾ In addition, oxidative DNA damage and activation of the poly(ADP-ribose) polymerase/p53/apoptosis inducing factor pathway were blocked by HGF administration, with the result that the cornu ammonis subregion 1 (CA1) neurons of the hippocampus were protected from apoptotic cell death after transient forebrain ischemia in rats.²³⁾ These findings indicate that HGF provides a potential therapeutic approach for cerebral ischemia.

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4. TROPHIC FACTORS

The neurotrophins consisting of brain derived neurotrophic factor (BDNF), neurotrophin-3, neurotrophin-4, and nerve growth factor (NGF) are a group of secreted proteins that perform major roles in synaptic regulation, differentiation and survival. The biological effects of each neurotrophin are exerted by the activation of the three different members of the tropomyosin-related kinase (Trk) receptors, TrkA, TrkB and TrkC. The neurotrophins BDNF and NGF have been extensively studied with respect to their positive effect on neuron survival in neurodegenerative disorders and nerve injuries of the CNS.^{24–26)} BDNF and NGF protected hippocampal progenitor cells from staurosporine-induced apoptosis by stimulating the phosphatidylinositol-3-kinase/Akt pathway through the Trk receptors.²⁷⁾ Exogenous treatment of BDNF or NGF in cultured hippocampal neurons attenuated glutamate-induced neurotoxicity by increasing the activity of antioxidant enzymes and decreasing the elevation of intracellular calcium ion.²⁸⁾ Furthermore, histone deacetylase inhibitors have been shown to be neuroprotective against acute traumatic brain injury in rodent models, and treatment of these inhibitors preserves the expression level of NGF and the activity of the TrkA survival pathway, resulting in reduction of cortical contusion volume and inflammation in a rat model of traumatic brain injury.²⁹⁾ In addition, studies using neurotrophin-deficient mice have shown that BDNF and neurotrophin-3 are crucial for the survival of developing neurons in the cerebellum.^{30,31)} Recently, the selective TrkB agonists 7,8-dihydroxyflavone and deoxydunin were reported to elicit receptor dimerization and autophosphorylation and induce the activation of the downstream signaling targets of TrkB such as ERK and Akt.^{32,33)} 7,8-Dihydroxyflavone reduced kainic acid-induced apoptosis or infarct volume after MCAO in wild type but not in TrkB-deficient mice. Furthermore, systemic administration of 7,8-dihydroxyflavone was able to improve hippocampus-dependent learning and memory in a mouse model of Alzheimer's disease.³⁴⁾

In addition to BDNF and NGF, ciliary neurotrophic factor (CNTF) and glial cell-line derived neurotrophic factor (GDNF) also have a neuroprotective effect against neurodegeneration.³⁵⁾ Intracerebral administration of CNTF sustained the survival of striatal output neurons in a rat model of Huntington's disease whereas BDNF, NGF or neurotrophin-3 did not.³⁶⁾ Furthermore, intravitreal injection of CNTF protected retinal ganglion cells through the Janus kinase (JAK)/STAT pathway in an ocular hypertensive glaucoma model.³⁷⁾ Autologous transplantation of mesenchymal stem cells expressing GDNF in a cynomolgus monkey model of Parkinson's disease improved the motor performance and protected the dopaminergic neurons in the striatum.³⁸⁾ Adeno-associated viral vector-mediated gene delivery of GDNF or BDNF provided significant neuroprotection of striatal interneurons in a quinolinic acid lesion model of Huntington's disease.³⁹⁾ As shown above, single or combination treatment of trophic factors or their receptor agonists seems to be a promising strategy for neuroprotection against neurodegenerative disorders and CNS injuries.

5. APOLIPOPROTEIN E-CONTAINING LIPOPROTEIN

The expression of apolipoprotein (apo) E is highest in the liver and second highest in the brain. In the brain, glia secrete lipoproteins and play important roles in lipid metabolism and transport. It has been reported that glia-derived lipoproteins are high density lipoprotein-like particles in terms of size and density and contain apo E, apo J, apo D, but not apo A1.^{40,41)} Although apo A1 is the main apo constituent of high density lipoproteins in plasma, apo E is the major apolipoprotein in cerebrospinal fluid. It has been reported that apo E-containing lipoproteins secreted from glia have a neuroprotective effect against trophic factor-withdrawal- and excitotoxicity-induced apoptosis in primary cultured retinal ganglion cells.^{42–44)} Furthermore, intravitreal injection of apo E-containing lipoproteins protected retinal ganglion cells from degeneration in retinæ of glutamate/aspartate-transporter deficient mice, a model of normal tension glaucoma.⁴⁴⁾ Low density lipoprotein receptor-related protein 1 (LRP1), which is a member of the low density lipoprotein receptor family, mediates the protective effect of apo E-containing lipoproteins and initiates an intracellular signaling pathway involving phospholipase C γ 1,

Fig. 1. Proposed Pathway of LRP1-Mediated Neuroprotection from Apoptosis Induced by Glutamate

Binding of apo E-containing lipoproteins to LRP1 activates phospholipase C γ 1 (PLC γ 1) and produces diacylglycerol (DAG) and inositol trisphosphate (IP₃) from phosphatidylinositol bisphosphate (PIP₂). DAG stimulates protein kinase C δ (PKC δ) and subsequently inactivates glycogen synthase kinase 3 β (GSK3 β) by phosphorylation. In addition, the formation of an LRP1-NMDA receptor complex is induced by apo E-containing lipoproteins and blocks intracellular Ca²⁺ overload caused by glutamate. Thus, neurons are protected from degeneration.

protein kinase C δ and glycogen synthase kinase 3 β . In addition, binding of apo E-containing lipoproteins to LRP1 facilitates the formation of LRP1 and NMDA receptors and prevents intracellular calcium overload through NMDA receptors (Fig. 1). It has also been reported that other LRP1 ligands such as α 2-macroglobulin, tissue plasminogen activator and matrix metalloproteinase-9 transactivate Trk receptors through a Src family kinase pathway in PC12 cells, cerebellar granular neurons and sensory neurons. Therefore, the authors concluded that various LRP1 ligands might have neurotrophic activity for neurite outgrowth, development and survival.^{45,46)}

6. PROTHYMOSIN α

Prothymosin α is an acidic and a hydrophilic nuclear protein that is broadly expressed in a wide variety of mammalian cells.⁴⁷⁾ It was originally isolated as the precursor of thymosin α 1 from rat thymus and is considered as a hormone-like thymic factor.⁴⁸⁾ However, prothymosin α was isolated from the serum-free conditioned medium of cortical neuron cultures and protected the cultured neurons from necrosis through the activation of the G_{i/o}-coupled receptor with phospholipase C and protein kinase C β _{II}.⁴⁹⁾ In addition, systemic administration of recombinant prothymosin α blocked both necrosis and apoptosis following the focal ischemia induced by MCAO.⁵⁰⁾ Recently, Halder *et al.*⁵¹⁾ demonstrated that the active core peptide domain P₃₀ at amino acids 49–78 of prothymosin α had the original neuroprotective activity of prothymosin α against ischemic damage *in vitro* in primary cultured cortical neurons, and *in vivo* in the retina and the brain. These findings indicate that prothymosin α seems to be a promising candidate for the treatment of stroke.

7. ERYTHROPOIETIN

Erythropoietin (EPO) is a glycoprotein expressed in the fetal liver, and is then predominantly produced in the kidney shortly after birth.⁵²⁾ It was originally characterized as a humoral regulator of erythropoiesis in the maturation and the proliferation of erythroid progenitor cells. EPO and its receptor are also expressed in the brain. Growing evidence about EPO suggests that EPO acts as a potent neuroprotectant against apoptosis and/or inflammation induced by nerve injuries or disorders.^{53–55)} Morishita *et al.*⁵⁶⁾ reported that the administration of EPO protected primary cultured cortical and hippocampal neurons from glutamate-induced neurotoxicity. It has also been reported that infusion of exogenous EPO into the lateral ventricles of gerbils attenuates cerebral ischemia-induced neuron loss in the CA1 of the hippocampus and learning impairment.⁵⁷⁾ Furthermore, EPO rescued cultured hippocampal neurons from nitric oxide-induced but not NMDA receptor-mediated neuronal death, suggesting that the neuroprotective effect of EPO may be exerted by reducing the free radical formation mediated by nitric oxide. Viviani *et al.*⁵⁸⁾ reported that neuronal apoptosis induced by the neurotoxicant trimethyltin was inhibited by the EPO treatment in the primary cultured hippocampal neurons since EPO promoted the transcription of the cAMP responsive element binding protein and the expression and the production of BDNF. The neuroprotective mechanisms by EPO have not been fully elucidated although the details of the mechanisms of the neuroprotection

by EPO have been investigated by multiple groups. Upon the binding of EPO to the EPO receptor dimer, JAK-2 is activated by autophosphorylation in neurons. The activation of JAK-2 causes several intracellular signal transductions involving nuclear factor- κ B, STAT-5, mitogen activated protein kinase and phosphatidylinositol-3-kinase, and then promotes the expression of neuroprotectants such as bcl-2 and bcl-XL (see details in reviews^{55,59–61)}). In addition, based on the large number of positive findings of EPO as a neuroprotectant in cultured cells, animal models and also human disorders,⁶²⁾ EPO is one of the most promising neuroprotective agents for the treatment of human brain disorders. Therefore, clinical trials with EPO are ongoing for traumatic brain injury, preterm neuroprotection and hypoxic-ischemic encephalopathy (see at <https://clinicaltrials.gov>).

8. TISSUE INHIBITOR OF METALLOPROTEINASE 1

The family of tissue inhibitors of metalloproteinase (TIMP) consists of four different members, TIMP1, 2, 3 and 4. Since TIMPs inhibit the activity of matrix metalloproteinases, the balance between matrix metalloproteinase and TIMP is important in the remodeling of extracellular matrix associated with cell migration, growth and survival.⁶³⁾ The expression of TIMP1 mRNA was significantly increased 24h after ischemic preconditioning induced by short occlusion of the middle cerebral artery, corresponding to the occurrence of significant ischemic tolerance.⁶⁴⁾ TIMP1 transgenic mice exhibited a significant reduction of the neuronal damage compared with wild-type mice after focal cerebral ischemia and traumatic brain injury.⁶⁵⁾ Furthermore, the serum TIMP1 level was associated with severity and mortality in patients with severe traumatic brain injury and may be a potential prognostic biomarker of mortality after traumatic brain injury.⁶⁶⁾ In addition to the activation of the JAK/STAT pathway in the neuroprotective mechanism of EPO, EPO also promotes significant cell survival associated with the increase of TIMP1 activity against hypoxic ischemia *in vitro* and *in vivo*.⁶⁷⁾ Furthermore, TIMP1 secreted from adipose stromal cells showed a neuroprotective effect against apoptosis induced by oxygen-glucose deprivation in primary cortical neuron cultures.⁶⁸⁾ Interestingly, astrocyte-derived TIMP1 not only protects primary cultured human neurons against human immunodeficiency virus-1-associated and staurosporine-induced neurotoxicity but also shows a neurotrophic effect in the anti-apoptotic pathway without the involvement of matrix metalloproteinase inhibition.⁶⁹⁾ Further studies are required to completely understand the detailed roles of TIMP1 in neuroprotection.

9. CONCLUSION

Endogenous neuroprotectants play crucial roles during development, maturation, maintenance and repair under normal and pathological conditions in the CNS as described above. Although we expect that neuroprotectants protect and cure disorders and injuries, they need to be delivered to the CNS through the blood–brain barrier or around the damaged area for neuroprotection. Thus, improvements in delivery systems or modifications of neuroprotectants are also required for treatments. We hope that ongoing and future studies investigating neuroprotectants and their mechanisms will develop

novel therapies for neurodegenerative disorders and traumatic injuries in the CNS.

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Conflict of Interest The authors declare no conflict of interest.

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