REVIEW

Neurotrophic Activity in Cytokine-activated Astrocytes

Kazunari Yoshida and Shigeo Toya

Department of Neurosurgery, School of Medicine, Keio University, Tokyo, Japan

(Received for publication on September 17, 1996)

Abstract. Accumulating evidence indicates that various neurotrophic factors (NTFs) exist and function in the brain. In the mature mammalian brain, NTF expression is exclusively restricted to neurons. However, astrocytes activated by various cytokines, including fibroblast growth factor and interleukin-1β, produce a significant amount of nerve growth factor (NGF) in vitro. Furthermore, non-NGF type NTF expression in astrocytes is also activated by the cytokines. The cytokines also enhance both release of ciliary neurotrophic factor from and expression of high-molecular weight basic fibroblast growth factor (FGF) in astrocytes. In the early phase following brain injury, cytokine-activated astrocytes rescue the damaged neurons via NTFs and other biologically active molecules. (Keio J Med 46 (2): 55-60, June 1997)

Key words: astrocyte, cytokine, neurotrophic factor, cell adhesion molecule

The recent identification of various neurotrophic factors (NTFs) in the brain suggests that neurons in the central nervous system (CNS) require neurotrophic support as well as neurons in the peripheral nervous system (PNS).1-3 Nerve growth factor (NGF) is a classical NTF which was originally found as a neurite-promoting and survival factor for peripheral sensory and sympathetic neurons.4,5 In the middle of the last decade, NGF was shown to exist and function in the brain.6 Recently, several NGF-related neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), NT-4/5, NT-6, have been identified.7-12 These NTFs are produced by target-cells of neurons which are sensitive to a distinct NTF, and are transported to the soma by the retrograde axonal transport mechanism. From the mechanism of neurotrophic action, these NTFs are generically classified as a target-derived growth factor (TDGF). The cholinergic neurons in the mammalian basal forebrain are NGF-sensitive neurons in the CNS.13 For example, the septohippocampal cholinergic pathway is a well-characterized model to explain the neurotrophic function of a TDGF-type NTF in the CNS. NGF produced by hippocampal neurons supports the survival and function of septal cholinergic neurons. On the other hand, various multifunctional growth factors and cytokines, including acidic and basic fibroblast growth factors (aFGF and bFGF) and ciliary neurotrophic factor (CNTF), possess neurotrophic activity for the CNS neurons.14-16 Expression of these neurotrophic cytokines in the normal adult brain is also largely neuronal. Although in the normal brain the neurotrophic function of FGFs and CNTF, which are non-secretory proteins lacking a signal sequence,17,18 remains unknown, previous findings indicate that various NTFs are produced and act in the brain not only in the mature brain, but also during the normal developmental stage and the recovery stage following brain injury.19

Astrocytes, which are stromal cells peculiar to the nervous system, have unique neurotrophic effects which are considered to be mediated by NTFs, cell adhesion molecules, and the extracellular matrix. We have previously demonstrated that astrocytes suppress the proliferation of neuroblasts and promote the neurite-elongation and survival of CNS neurons in vitro.20 Accumulating evidence indicates that astrocytes produce NTFs, which exclusively express in the mature CNS neurons, under a pathological condition. In this review article, we describe the neurotrophic activity of astrocytes activated by various cytokines and their putative biological function.
NGF Expression in Astrocytes

As previously described, NGF expression in normal adult brain is largely neuronal. However, astrocytes produce a significant amount of NGF in vitro. Although NGF synthesis and secretion by resting astrocytes after reaching confluency is very small, astrocytes just after passage produce a high level of NGF. Mechanical stimulation during the cell-passage procedure might stimulate astrocytes to produce NGF. Moreover, NGF synthesis in astrocytes is cooperatively up-regulated by various cytokines, i.e., αFGF, βFGF, interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), transforming growth factor-β1 (TGF-β1), epidermal growth factor (EGF) and transforming growth factor-α (TGF-α). These growth factors and cytokines possess variable potent biological activity for neurons, astrocytes and other mesenchymal cells in the CNS. However, many of the biological activities might be masked or suppressed in the normal brain. A high amount of FGFs exists in normal brain, and these multipotent growth factors stay inside neurons due to the lack of a signal sequence, which is essential for secretion. However, FGFs might be released from damaged neurons. IL-1β and TNF-α are products of microglia activated following brain injury. Disruption of the blood-brain barrier may allow an influx of cytokines into brain, including TGF-β1, which are abundant in platelets. Thus, injury-induced cytokines activate astrocytes to secrete NGF. NGF which is produced by activated astrocytes may rescue the damaged NGF-sensitive neurons following brain injury. However, NGF should be provided by target cells of NGF-sensitive neurons to support the normal neuronal function. NGF produced by activated astrocytes ameliorates the damaged NGF-sensitive neurons only during the acute phase of brain injury. If neurotrophic support from target cells does not recover during the acute phase, the damaged neural network cannot be reconstructed. The proposed function of NGF produced by cytokine-activated astrocytes is schematically shown in Figure 1.

Non-NGF Type Neurotrophic Activity in Cytokine-activated Astrocytes

Activated astrocytes produce a significant amount of NGF. However, the population of NGF-sensitive neurons in the CNS is very small. A large number of CNS neurons should depend on the non-NGF type NTFs. We speculate that the cytokine-activated astrocytes may produce other NTFs besides NGF. To investigate the novel NTF, we examined the neurotrophic activity in astrocyte-conditioned medium (ACM). NGF enhances the choline-acetyltransferase (CAT) activity in cultured cholinergic neurons derived from rat medial septum. At first, we examined the effect of ACM on septal cholinergic neurons. ACM was found to enhance the CAT expression in cultured septal cholinergic neurons derived from rat embryo. ACM prepared from astrocytes pretreated by αFGF, IL-1β, TNF-α, TGF-β1 (S-ACM) has a more potent effect on septal cholinergic neurons than ACM. These NTFs ameliorate the damaged neurons during the acute and subacute phase of brain injury. Thus, injury-induced cytokines should be provided by the target neurons of NGF-sensitive septal cholinergic neurons at the final stage of reconstruction. If NTFs produced by activated astrocytes act successfully, the septo-hippocampal pathway may regenerate.
cholinergic neurons from the brainstem and spinal cord was examined, because these neurons are not NGF-sensitive. ACM slightly enhanced CAT expression in both brainstem and spinal cholinergic neurons, and the effect of S-ACM was shown to be more potent than ACM, suggesting that cytokine-activated astrocytes release a non-NGF type cholinergic NTF(s). Furthermore, we have demonstrated that the extract of cytokine-activated astrocytes displays a novel neurotrophic activity for pontine cholinergic neurons.29

In addition to NGF, astrocytes are shown to express various secretory NTFs and neurotrophic cytokines, such as BDNF, NT-3, NT-4/5, IL-3, IL-6, leukemia inhibitory factor (LIF) and glial cell line-derived neurotrophic factor (GDNF).30–36 These NTFs and neurotrophic cytokines might contribute to neurotrophic activity in cytokine-activated astrocytes. Among the NTFs expressed in astrocytes, LIF is the only NTF in which expression is enhanced by IL-1β, TNF-α and TGF-β1. However, S-ACM has a distinct neurotrophic activity from LIF and NGF, suggesting that cytokine-activated astrocytes express a novel cholinergic NTF.

FGF and CNTF in Cytokine-activated Astrocytes

Reactive astrocytes express a significant amount of FGF and CNTF, which seem to be non-secretory proteins because lack of the signal sequence.37,38 To evaluate the role of FGF and CNTF in cytokine-activated activated astrocytes, we examined the effect of cytokines which enhance NGF-expression in astrocytes on bFGF and CNTF expression in astrocytes.39 A significant amount of CNTF exist in cultured astrocytes under usual culture conditions, however, cytokines, including IL-1β and TNF-α, have no effect on CNTF expression in astrocytes. Astrocytes also express CNTF-receptor (CNTF-R) in vitro. Although the mechanism of CNTF release from astrocytes is unknown, it must be released to display its biological activity. However, CNTF was never detected in a culture medium of astrocytes cultured under normal conditions. We hypothesized that CNTF release from astrocytes by an unknown mechanism might be rapidly sequestered by the CNTF-R expressed in astrocytes. To confirm our hypothesis, we cleaved the CNTF-binding domain of CNTF-R by disrupting glycosylphosphatidylinositol anchor of CNTF-R on astrocytes by phosphatidylinositol-specific phospholipase C (PI-PLC). We could detect a low but significant level of CNTF immunoreactivity in culture medium of PI-PLC treated astrocytes by two-site ELISA. Furthermore, culture media of astrocytes treated with IL-1β, TNF-α and EGF contain a high amount of CNTF, indicating that rapid sequestration of CNTF by CNTF-R is the reason why CNTF was never detected in astrocyte-culture medium, and that these cytokines enhance the release of CNTF from astrocytes.

CNTF, which was initially identified based on its ability to support the survival of parasympathetic neurons of ciliary neurons,40 has been shown to affect a wide range of neurons and neuronal precursors.41 In addition to its neurotrophic property, CNTF has a broad spectrum of biological actions. For example, CNTF induces acute-phase protein expression in hepatocytes.42 CNTF expression in the normal adult brain is very low. However, reactive astrocytes express a significant amount of CNTF following brain injury. Accumulating evidence indicates that CNTF is an injury-induced factor. We have demonstrated the release of this multipotent neurotrophic factor from cytokine-activated astrocytes. Although the long-term exposure of CNTF to the nervous tissue may result in adverse effects, CNTF released from cytokine-activated astrocytes plays an important role in neuronal plasticity especially during the acute or subacute phase following brain injury.

We also examined bFGF expression in cytokine-activated astrocytes. Western blot-analysis revealed the expression of 18, 22 and 24-kD bFGF isoforms in astrocytes. Translation of a single bFGF mRNA initiates from an AUG codon yielding an 18-kD isoform, and initiation from CUG codons which locate at the upstream of the AUG initiating codon produces high molecular weight (HMW) bFGF isoforms (22 and 24-kD isoforms).43 The HMW isoforms of bFGF have a nuclear targeting sequence, which is necessary for translocation of bFGF from the cytoplasm to the nucleus. IL-1β, TNF-α and EGF selectively enhance the expression of HMW-bFGF isoforms, but not the 18-kD isoform. Immunocytochemical analysis demonstrated that bFGF immunoreactivity increases in cytoplasm of astrocytes by cytokine treatment followed by translocation of bFGF to the nucleus in 3 days. Translocation of HMW bFGF isoforms may induce various gene expression in astrocytes. Thus, HMW bFGF isoforms expressed in cytokine-activated astrocytes might enhance the neurotrophic activity in astrocytes themselves by an autocrine mechanism.

FGFs, which were originally purified from the brain and the pituitary gland as a potent mitogen for fibroblasts, have angiogetic and neurotrophic activity besides mitogenic activity on various cell types.44,45 As we have demonstrated, FGFs are a potent mitogen and NGF-inducer for astrocytes.22 FGFs are produced by astrocytes themselves. FGFs produced by astrocytes may act as an autocrine growth factor for astrocytes and a paracrine neurotrophic and angiogenic factor for neurons and vascular endothelial cells, respectively. FGFs produced by activated astrocytes promote the reconstruction of a damaged neural network directly via their own neurotrophic activity and indirectly by activating various neurotrophic activity in astrocytes.

On the other hand, bFGF was shown to have tumori-
genic potential. Over-expression of 18-kD bFGF isoform results in malignant transformation of fibroblasts. However, there is no evidence of tumorigenicity of HMW bFGF isoforms. Moreover, HMW bFGF isoforms, but not the 18-kD isoform, were shown to promote regeneration of rat liver after heparectomy. Our results demonstrate that the cytokines, including IL-1β, selectively enhance HMW bFGF isoform expression in astrocytes, suggesting that HMW bFGF isoforms may play an important role in regeneration of the damaged CNS.

Neural Plasticity and Cytokine-activated Astrocytes

We have described the NTFs and neurotrophic activity of cytokine-activated astrocytes. Cell adhesion molecules and the extracellular matrix may also play an important role in neurotrophic activity in cytokine-activated astrocytes. Our preliminary experiment revealed that neural cell adhesion molecule (NCAM) expression in astrocytes is enhanced by TGF-β1. Cytokine-activated astrocytes seem to promote reconstruction of the damaged neuronal network following brain injury via NTFs and other biologically active molecules. Astrocytes have potent neurotrophic property especially in vitro. Accumulating evidence indicates that neurotrophic factor expression in astrocytes occurs following brain injury in vivo. We have detected a significant amount of NGF in the cerebrospinal fluid (CSF) of human patients following neurosurgery and subarachnoid hemorrhage, indicating that NGF synthesis in the human CNS is enhanced following brain damage. Non-NGF type neurotrophic activity is also accumulated in CSF following neurosurgery or subarachnoid hemorrhage. Activated astrocytes are a candidate of the source of NGF and non-NGF type neurotrophic activity in human CSF.

Glucocorticoids are one of the most efficient neuroprotective reagents especially for patients with brain edema. However, glucocorticoids suppress NGF expression and NGF-mediated neurotrophic activity in cytokine-activated astrocytes. Although the effects of glucocorticoids in human patients is unquestionable, the effects of glucocorticoids on the neurotrophic activity in activated astrocytes in human patients should be evaluated.

Astrocytes are a major component of the glial scar formed following brain injury. Because regenerating axons cannot penetrate the glial scar, these astrocytes are considered to prevent the neural regeneration in the CNS. So-called reactive astrocytes in the glial scar are the consequence of sequential events following brain injury. The activated astrocytes described in this manuscript are biological active astrocytes during the acute and subacute phase of brain injury. As shown in Figure 2, various NTFs produced by activated astrocytes prevent neuronal death and promote the survival and neurite-reoutgrowth of damaged neurons only during the early period following brain injury. In addition to astrocytes, microglia and fibroblasts might produce NTFs in vivo, because fibroblasts and microglia activated by FGFs and lipo-poly-saccharide, respectively, produce NGF.

Fig 2 The proposed mechanism of cytokine-activation of astrocytes following brain injury. Astrocytes are activated by various cytokines released from damaged neurons, activated microglia, or blood. NGF, FGFs, CNTF and other NTFs released from activated astrocytes ameliorate the damaged neurons in the early stage of brain injury. Activated microglia and fibroblasts are also shown to produce NGF. NGF from these cells act as NTFs in the case of brain injury. So-called "reactive astrocytes" no longer actively produce NGF.

Activated astrocytes play an important role in the process of neuronal plasticity following brain injury. However, it is usually very difficult to achieve the sufficient reconstruction of the damaged neural network in the CNS. For instance, re-myelination of damaged axon in the CNS hardly occurs, because oligodendrocytes, which are the myelin-forming cells in the CNS, do not proliferate in the adult brain, while schwann cells in the PNS actively proliferate following nerve injury. Many problems remain to be solved to reconstruct the damaged neural
network in the CNS. NTFs produced by activated astrocytes play one of key roles in the healing process. The CNS is composed by many kinds of neurons with variable properties. There should be many kinds of NTFs besides NTFs described in this manuscript. Cytokine-activated astrocytes are one of the best source of the unknown NTFs.

Acknowledgement: This work was partially supported by grants form The Ministry of Education, Science and Culture, Japan, and Keio University.

References

3. Thoenen H: Neurotrophins and neuronal plasticity. Science 1995; 270: 593–598
15. Walicke PA: Basic and acidic fibroblast growth factors have trophic effects on neurons from multiple CNS regions. J Neurosci 1988; 8: 2618–2627
34. Zafra F, Lindholm D, Castrén E, Hartikka J, Thoenen H: Regulation of brain-derived neurotrophic factor and nerve growth
47. Presta M, Statuto M, Rusnati M, Dell’Era P, Ragnotti G: Characterization of a Mr 25,000 basic fibroblast growth factor form in adult, regenerating, and fetal rat liver. Biochem Biophys Res Commun 1989; 164: 1182–1189