Expression of Nerve Growth Factor and trkA After Transient Focal Cerebral Ischemia in Rats

Tsong-Hai Lee, MD, PhD; Hiroyuki Kato, MD, PhD; Sien-Tsong Chen, MD; Kyuya Kogure, MD, PhD; Yasuto Itoyama, MD, PhD

Background and Purpose—In vitro studies have shown that nerve growth factor (NGF) is protective to cortical neurons against various insults. However, the role of NGF in relation to its high-affinity trkA receptor in the cortical neurons has not been well discussed. In this experiment, we studied the possible involvement of the NGF/receptor system in the ischemic injury of cortical neurons after focal cerebral ischemia in rats.

Methods—Male Wistar rats received right middle cerebral artery occlusion of 90 minutes’ duration. The rats were decapitated at different reperfusion time points: hour 4 and days 1, 3, 7, and 14 of recirculation. Brain sections at the level of striatum were immunostained against NGF, trkA, glial fibrillary acidic protein (GFAP), and stress protein HSP70. Double immunostaining against NGF and GFAP was also performed. Optical density of NGF immunoreactivity in the ischemic and nonischemic cortices was compared between sham-control and ischemic animals.

Results—In the sham-control rats, NGF immunoreactivity was present in the cortical and striatal neurons. However, beginning at hour 4 after recirculation, there was a significant decrease of NGF in the ischemic cortex and striatum. Beginning at day 1, NGF was absent completely in the infarcted striatum and cortex. However, in the peri-infarct penumbra area, despite a decrease in NGF at hour 4 and day 1, NGF recovered beginning at day 3 and returned almost to the sham-control level at day 14. In the nonischemic cortex, NGF increased beginning at hour 4, peaked at day 7, and returned almost to the sham-control level at day 14. The trkA and HSP70 immunoreactivities were not present in the sham-control cortex. However, trkA was induced at hour 4 in the ischemic cortex and at days 1 and 3 in the peri-infarct penumbra cortex. The HSP70 was induced at days 1 and 3 in the peri-infarct penumbra area. Double immunostaining showed that the number of GFAP-positive cells increased gradually, and NGF immunoreactivity in the GFAP-positive cells became gradually intense after ischemia.

Conclusions—Our study demonstrated a temporal profile of NGF and trkA in the ischemic cortex and NGF expression by reactive astrocytes. Our data suggest that the NGF/receptor system may play a role in the astrocyte/neuron interaction under certain pathological conditions, such as focal cerebral ischemia. (Stroke. 1998;29:1687-1697.)

Key Words: nerve growth factors | cerebral ischemia, focal | immunohistochemistry | rats

Neurotrophic factors are known to be critically involved in neurite outgrowth and cell survival.1 In the central nervous system, neurons can express genes of various neurotrophic factors. Of these, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3) are known to be present at the highest level in the hippocampal formation and cerebral cortex.2-6 The NGF, BDNF, and NT-3 can interact with the low-affinity receptor (p75) with similar binding affinity. However, it is the high-affinity form (trkA for NGF, trkB for BDNF, and trkC for NT-3) that acts as a signal-transducing receptor for these neurotrophins.7

NGF and BDNF, but not NT-3, can enhance the survival and differentiation of cultured embryonic basal forebrain cells.1,8,9 After various brain injuries, such as cerebral ischemia or hypoglycemic coma, NGF and BDNF mRNAs increase but NT-3 mRNA decreases in the hippocampal neu

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injection of colchicine,19 NGF protein increases in the neocortex and hippocampal formation. NGF protein can also increase in the hippocampal formation after forebrain ischemia.20 However, the role of NGF in relation to its high-affinity trkA receptor in the ischemic cortex remains unclear.

In the present study, we used 70-kDa heat shock protein (HSP70) as a marker of cell injury21 to identify injured regions in the ischemic cortex and studied the possible involvement of NGF and trkA in the ischemic neuronal injury after focal cerebral ischemia. We also used double immunostaining against NGF and glial fibrillary acidic protein (GFAP) to study the possible role of reactive astrocytes after cerebral ischemia.

Materials and Methods

Male Wistar rats of SPF strain, aged 10 to 12 weeks and weighing 15.5 to 16.5 mm of nylon surgical thread (depending on the weight of the rat) was inserted into the right internal carotid artery. Insertion of the thread was made round by heating. Blunting the distal end (which was made round by heating) met resistance, the proximal end of the thread was tightened at the carotid bifurcation. The right common carotid artery was then permanently ligated, and anesthesia was discontinued. After a 90-minute occlusion of the right MCA, the nylon surgical thread was removed to allow complete reperfusion of the ischemic area through the anterior and posterior communicating arteries. Using this modified method, approximately 15.5 to 16.5 mm of nylon surgical thread (depending on the weight of the rat) was inserted into the right internal carotid artery. Insertion of nylon surgical thread into the right internal carotid artery until the distal end may also prevent the easy perforation of the vessel wall. Blunting the distal end (which was made round by heating) met resistance, the proximal end of the thread was tightened at the carotid bifurcation. The right common carotid artery was then permanently ligated, and anesthesia was discontinued. After a 90-minute occlusion of the right MCA, the nylon surgical thread was removed to allow complete reperfusion of the ischemic area through the anterior and posterior communicating arteries. Insertion of nylon surgical thread into the right internal carotid artery until the distal end may also prevent the easy perforation of the vessel wall. Blunting the distal end (which was made round by heating) met resistance, the proximal end of the thread was tightened at the carotid bifurcation. The right common carotid artery was then permanently ligated, and anesthesia was discontinued. After a 90-minute occlusion of the right MCA, the nylon surgical thread was removed to allow complete reperfusion of the ischemic area through the anterior and posterior communicating arteries.

During ischemia, rectal temperature was monitored in all the animals and was maintained at approximately 37°C with a heating pad and an overhead lamp. After restoration of blood flow, the animals were allowed to recover at ambient temperatures (21°C to 23°C). Reperfusion time points were 4 hours and 1, 3, 7, and 14 days (n=3 at each time point). Focal cerebral ischemia of 90 minutes’ duration is known to cause irreversible brain injury in the ischemic cerebral cortex and striatum.22 We included in the study those animals that exhibited left hemiparesis with upper limb dominance and severe infarct in the ischemic striatum and cortex. Sham-control animals (n=4) were prepared in the same way except for the insertion of a 4–0 nylon surgical thread into the right internal carotid artery. A total of 26 rats were used in the experiment. The procedures used in this study were approved by the Institutional Animal Committee.

Animals were decapitated at different reperfusion time points, as previously indicated. Brains were dissected out, frozen in powdered dry ice, and stored at −80°C. Coronal sections (20 μm) at the level of striatum were cut on a cryostat at −18°C, collected on slide glasses coated with Vectabond (Vector Labs), and stored at −80°C until immunostaining.

Immunostaining against NGF,24–27 trkA, GFAP, and HSP70 in fresh-frozen brain sections was performed by the avidin-biotin peroxidase (ABC) method using a kit (PK-6101 for NGF and trkA and PK-6102 for GFAP and HSP70; Vector Labs). First, fresh-frozen sections were fixed in ice-cold acetone for 30 minutes and air-dried. Then, sections were rinsed in 0.01 mol/L phosphate buffer containing 0.15 mol/L NaCl (PBS) (pH 7.2). After blocking with 10% normal serum (normal goat serum for NGF and trkA and normal horse serum for GFAP and HSP70) for 2 hours, sections were washed and incubated for 12 to 15 hours at 4°C with a rabbit polyclonal antibody against mouse NGF (1/500) (Chemicon), a rabbit polyclonal antibody against trkA (1/200) (Oncogene Science Inc), a mouse monoclonal antibody against human brain GFAP (1/200) (Amersham), or a mouse monoclonal antibody against HSP70 (1/200) (Amersham) diluted in PBS. Endogenous peroxidase was blocked for 30 minutes with 0.1% H2O2 and 100% methanol. The sections were then washed and incubated for 3 hours with a biotinylated anti-rabbit IgG antibody made in goat for NGF (1/500) and trkA (1/200) and anti-mouse IgG antibody made in horse for GFAP and HSP70 (1/200) in PBS, followed by incubation for 1 hour with an avidin–biotin–horseradish peroxidase complex (1/100). Staining was developed with 3,3′-diaminobenzidine tetrahydrochloride (DAB) (0.5 mg/mL in 50 mmol/L Tris-HCl buffer, pH 7.6) in the presence of 0.02% H2O2. All the incubations were done at room temperature except for the primary antibody. Double immunostaining for NGF and GFAP was performed according to our previous study.26 The sections were first processed for NGF as earlier described. After reaction with DAB, the sections were washed briefly in PBS, incubated for 30 minutes in excess of avidin and then, for 30 minutes in excess of biotin (Vector Labs). The rest of the procedure was the same as described earlier for GFAP. The first primary antibody was reacted with DAB, which gave a brown reaction, and the second primary antibody was reacted with DAB solution plus nickel chloride (2 mg/mL), which gave a blue-black reaction. To ascertain the specificity of double immunostaining, the primary antibody was omitted during the first and/or second stage of the immunostaining.

Immunohistochemical controls against NGF and trkA were done by omitting the primary antibody and by using preimmune rabbit IgG instead of the primary antibody. Immunohistochemical control against GFAP and HSP70 was done by omitting the primary antibody. The specificity of polyclonal antibody against NGF was proved in our previous study,25 which demonstrated that this anti-NGF antibody recognized NGF antigen specifically and did not cross-react with BDNF and NT-3. The specificity of polyclonal antibody against trkA was proved by previous studies.28,29 The specificity of mouse monoclonal antibody against GFAP and HSP70 for rat brain tissue was described by Amersham.

Optical density of NGF immunoreactivity in the ischemic and nonischemic cerebral cortex areas of the MCA territory was detected by an Image Analyzer (Kontron, Karl Zeiss) and was calculated using the following equation: −log (optical density of detected cerebral cortex/9 optical density of background).25 The background density was detected on the sections immunostained without the primary antibody. The data between the sham-control and ischemic animals at each time point in the ischemic and nonischemic cortex were statistically compared. Statistical analysis was performed with Student’s t test.

The ischemic area was defined as the area injured after occlusion of the right MCA and includes the peri-infarct penumbra area and the infarcted cortex and striatum. Because HSP70 is a marker of cell injury21 and is known to be expressed in the peri-infarct penumbra area,21,31 we used HSP70 immunostaining to define the location of the peri-infarct penumbra area. The nonischemic area comprised the left hemisphere and the area in the right hemisphere not injured after occlusion of the right MCA.

Results

After 90 minutes of right MCA occlusion, the ischemic area at each time point was confined to the cortex and striatum supplied by the right MCA and was not found in the ipsilateral anterior cerebral artery and posterior cerebral artery territories and contralateral hemisphere. This result suggested that our MCA occlusion model in rats had good reproducibility.

In the sham-control brains, NGF immunoreactivity was seen in the striatal neurons and in the cortical neurons of layers II–VI (Figure 1A), which was similar to the finding of
Figure 1. The NGF immunoreactivity is normally present in the sham-control cerebral cortex and striatum (A). At day 1 (B) after 90 minutes of right MCA occlusion, NGF immunoreactivity increases in the nonischemic cortex. At day 7 (C), NGF increases significantly and at day 14 (D), NGF returns almost to the sham-control level (A). Panels E, F, and G show the changes in NGF in the infarcted areas (arrowheads), the peri-infarct penumbra (such as the area marked by a rectangle), and the nonischemic areas (the left lower area). In the ischemic cortex and striatum where infarct has formed (arrowheads), beginning at day 1 after 90 minutes of right MCA occlusion, NGF immunoreactivity is absent. In the peri-infarct penumbra cortex where infarct has not formed, NGF recovers gradually and returns almost to the sham-control level at day 14 (G). However, in the cortical area where ischemic injury is not induced (lower left of the cortex), NGF immunoreactivity increases gradually with a peak at day 7 (F) and returns almost to the sham-control level (A) at day 14 (G). s indicates striatum; c, corpus callosum; p, parietal cortex; and i, infarcted cortex. Arrowheads indicate the margin of infarcted cortical area. Rectangle in panels E, F, and G indicates the peri-infarct penumbra area shown at a larger magnification in Figure 2. Bar in A is 200 μm.
A gradual increase of NGF immunoreactivity was observed in the nonischemic cortex beginning at hour 4 after 90 minutes of right MCA occlusion (Figure 1B for day 1). Beginning at day 3 (D) and 7 (E), NGF recovers gradually in the peri-infarct penumbra area. At day 14 (F), NGF recovers almost to the sham-control level in the cells of peri-infarct penumbra area. i indicates infarcted or ischemic cortex. Arrowhead indicates the margin of infarcted cortex. Bar in panel A is 50 μm.

Nishio et al. A gradual increase of NGF immunoreactivity was observed in the nonischemic cortex beginning at hour 4 after 90 minutes of right MCA occlusion (Figure 1B for day 1). Beginning at day 3, there was a significant increase of NGF with a peak at day 7 (Figure 1C). At day 14 (Figure 1D), NGF returned almost to the sham-control level (Figure 1A).

Beginning at hour 4 after 90 minutes of right MCA occlusion, however, a significant decrease of NGF immunoreactivity was found in the ischemic cortex and striatum. Beginning at day 1 (Figure 1E for day 1, Figure 1F for day 7, and Figure 1G for day 14), NGF immunoreactivity was lost completely in the ischemic cortex and striatum where infarct had formed.
In the peri-infarct penumbra cortex where infarct had not formed, NGF immunoreactivity recovered beginning at day 3 (Figure 1F for day 7) and returned almost to the sham-control level at day 14 (Figure 1G).

In the parietal cortical neurons, NGF immunoreactivity was normally present in the cell body of the sham-control cortex (Figures 2A and 3A). However, in the ischemic parietal cortex, NGF decreased beginning at hour 4 after 90 minutes of right MCA occlusion (Figure 2B). Beginning at day 1 (Figure 2C), there was a complete loss of NGF immunoreactivity in the area where infarct had formed. However, in the peri-infarct penumbra area, despite a decrease in NGF at hour 4 (Figure 2B) and day 1 (Figure 2C), NGF recovered gradually beginning at day 3 (Figure 2D) and returned almost to the sham-control level (Figure 2F). In the nonischemic parietal cortex, after 90 minutes of right MCA occlusion (Figure 2B). Beginning at day 1 (Figure 2C), there was a complete loss of NGF immunoreactivity in the area where infarct had formed.
minutes of right MCA occlusion, NGF immunoreactivity increased gradually beginning at hour 4 (Figure 3B), peaked at day 7 (Figure 3E), and returned almost to the sham-control level (Figure 3A) at day 14 (Figure 3F).

The immunostaining of trkA showed that trkA immunoreactivity was not present in the cortical neuron of sham-control brain (Figure 4A) but was present in the interneurons of the striatum, which was similar to the study of Sobreviela et al.28 However, beginning at hour 4 (Figure 4B) after 90 minutes of right MCA occlusion, trkA was induced in the ischemic cortex but was not induced in the ischemic striatum (data not shown). At days 1 (Figure 4C) and 3 (Figure 4D), some trkA immunoreactivity was still present in the peri-infarct penumbra area. The trkA was not induced beginning at day 7 after ischemia in the ischemic cortex and at any time point in the nonischemic cortex (data not shown).

The immunostaining of HSP70 showed that HSP70 immunoreactivity was not present in the cerebral cortex and striatum of sham-control brain (Figure 5A) and at hour 4 (Figure 5B) after 90 minutes of right MCA occlusion. However, HSP70 was induced at days 1 (Figure 5C) and 3 (Figure 5D) in the peri-infarct penumbra area. The HSP70 was not induced beginning at day 7 after ischemia in the ischemic cortex and at any time point in the nonischemic cortex (data not shown).
cortex (data not shown). The immunostaining of trkA and HSP70 was done at the adjacent sections of the same animal.

Double immunostaining of NGF and GFAP showed that NGF immunoreactivity can be seen in both cortical neurons and GFAP-positive cells in the ischemic and nonischemic cortex. The NGF immunoreactivity increased beginning at days 3 to 7 in the ischemic peri-infarct penumbra cortex (Figure 6B for day 3 and 6D for day 7) and nonischemic cortex (Figure 6A for day 3 and 6C for day 7) after 90 minutes of right MCA occlusion. The GFAP-positive cells increased gradually at a larger amount in the ischemic cortex compared with the nonischemic cortex. The GFAP immunoreactivity also became gradually intense in individual reactive astrocyte after ischemia. The single immunostaining of GFAP (Figure 6E) and NGF (Figure 6F) in the ischemic cortex at day 7 was presented for comparison.

Figure 5. HSP70 immunoreactivity is not seen in the cortical neurons of sham-control brain (A) and at hour 4 (B) after 90 minutes of right MCA occlusion. However, at days 1 (C) and 3 (D), HSP70 is induced in the peri-infarct penumbra cortex. This induction of HSP70 is not seen beginning at day 7 in the ischemic cortex or at any time point in the nonischemic cortex (data not shown). i indicates infarcted cortex. Arrowhead indicates the margin of infarcted cortex. Bar in A is 100 μm.
In the immunohistochemical control study, no immunoreactivity was observed when sections were immunostained with the primary antibody omitted or with the preimmune IgG. The study of optic density showed a significant decrease of NGF immunoreactivity in the ischemic parietal cortex beginning 4 hours after 90 minutes of right MCA occlusion compared with the sham-control cortex ($P < 0.001$). However, in the nonischemic left parietal cortex, the optical density of NGF immunoreactivity showed a significant increase beginning at day 3 ($P < 0.05$), with a peak at day 7 ($P < 0.001$) and a return to almost the sham-control level at day 14 ($P < 0.01$) (Table).

**Discussion**

Previous immunohistochemical studies using rabbit polyclonal antibody against mouse NGF showed that NGF immunoreactivity was associated with fibers located in the hilum of dentate gyrus and in the CA2-CA3 areas of hippocampus and that it was not found in the neurons of hippocampal formation, cerebral cortex, and olfactory bulb. However, studies using enzyme-linked immunosorbent assay and northern blot analysis showed that the levels of NGF protein and mRNA were relatively high in normal hippocampal formation, cerebral cortex, and olfactory bulb. In situ hybridization studies also showed that NGF mRNA hybridized neurons were mainly present in the hippocampal formation and cerebral cortex. A recent immunohistochemical study using rabbit polyclonal antibody against mouse NGF demonstrated that NGF protein was located inside the cell body of neurons in widespread areas of brain, such as hippocampal formation, cerebral cortex, and striatum. Our study of NGF was compatible with in situ hybridization and recent immunohistochemical studies. It also demonstrated that NGF protein was present in the cell body of neurons (Figures 1A, 2A, and 3A).

Both NGF and basic fibroblast growth factor (bFGF) can protect cultured cortical and hippocampal neurons against hypoglycemic damage or iron-induced degeneration. In vivo studies also showed that treatment of NGF or bFGF can protect hippocampal CA1 neurons against delayed neuronal death. The expression of NGF, as shown in our study, and bFGF immunoreactivity can be enhanced in the cortical neurons after focal cerebral ischemia. Among the

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**Optic Density of NGF Immunoreactivity in the Ischemic and Nonischemic Parietal Cortex After 90 Minutes of Right Middle Cerebral Artery Occlusion**

<table>
<thead>
<tr>
<th>Optic Density</th>
<th>Sham</th>
<th>Hour 4</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>ischemic cortex Mean</td>
<td>0.0208</td>
<td>0.0053$^*$</td>
<td>-0.021$^*$</td>
<td>-0.0049</td>
<td>-0.0047$^*$</td>
<td>-0.0103$^*$</td>
</tr>
<tr>
<td>SD</td>
<td>0.0016</td>
<td>0.0016</td>
<td>0.0002</td>
<td>0.0007</td>
<td>0.0016</td>
<td>0.0020</td>
</tr>
<tr>
<td>nonischemic cortex Mean</td>
<td>0.0208</td>
<td>0.0244</td>
<td>0.0261</td>
<td>0.0284$^*$</td>
<td>0.0751$^*$</td>
<td>0.0306$^*$</td>
</tr>
<tr>
<td>SD</td>
<td>0.0039</td>
<td>0.0022</td>
<td>0.0005</td>
<td>0.0015</td>
<td>0.0006</td>
<td>0.0007</td>
</tr>
</tbody>
</table>

The ischemic cortex includes the peri-infarct penumbra and the infarcted cortex, which is injured after occlusion of the right middle cerebral artery. The nonischemic cortex indicates the cortex supplied by the left middle cerebral artery. $n = 3–4$ at each time point.

$P < 0.001$, $P < 0.01$, and $P < 0.05$ compared with the sham-control group. Student’s $t$ test was used for data comparison.
family of neurotrophic factors, NT-3 and BDNF can protect cultured rat cortical and hippocampal neurons against metabolic/excitotoxic insults. BDNF can also protect rat hippocampal neurons against ischemic cell damage. These data suggest that each neurotrophin may have its distinct role in the mechanism of neuronal injury. It is possible that in the central nervous system, neurons do not depend on a single neurotrophic factor for survival, and that a combination of two or more neurotrophic factors may be more effective in supporting the survival of neurons.

Neurotrophins are known to induce tyrosine phosphorylation of trk receptors in cultured rat embryonic brain. In rat forebrain cholinergic neuron, trkA gene and protein expression can be induced by intraventricular infusion of NGF. Under pathophysiological conditions, such as ischemic insult, trkB may be upregulated to favor an increased cellular response to injected BDNF. After systemic dexamethasone treatment, septal trkA can be upregulated maximally at hour 12, and it is suggested that this increase is regulated via the induction of NGF. Likewise, after a fimbria/ fornix transection, trkA is upregulated in neuronal and nonneuronal cells near the site of injury. Intraventricular application of NGF in rats with fimbria lesion also prevents loss of trkA mRNA in basal forebrain neurons. From these studies, we can see that neurotrophins can regulate the expression of their high-affinity receptors in response to various insults. This neurotrophin-mediated expression of high-affinity receptor is suggested to be an important feature of neurotrophins to regulate the activity of their responsive neurons.

The mRNAs for neurotrophins and their receptors are colocalized in the developing forebrain neuron, and this colocalization is suggested as a potential for autocrine and paracrine mechanisms of neurotrophin during development. In the developing dorsal root ganglion, trk is expressed in a time frame when it can mediate the response of neurons to neurotrophin; this expression of trk determines the dependence of neuron on neurotrophin during development. In our study, trkA is induced in the ischemic cortex where there is a significant reduction of NGF. Our study suggests that induction of trkA after ischemic injury may play an important role in regulating the responsiveness of ischemic neurons to NGF for survival.

HSP70 is not present in normal neurons but is inducible under various stressful conditions, including ischemia. Once HSP70 is induced, it may prevent certain proteins from being denatured and may protect cells from subsequent severe injury. In the study of focal cerebral ischemia, Kinouchi et al showed that HSP70 was induced in the peri-infarct penumbra area at days 1 and 2 of recirculation. Our study of 90-minute focal cerebral ischemia is in accordance with these reports and demonstrated that HSP70 is induced in the peri-infarct penumbra area at days 1 and 3 of recirculation. HSP70 is regarded as a marker of cell injury and is used to identify injured regions in the ischemic cortex. The decrease in NGF immunoreactivity in the peri-infarct penumbra area where HSP70 is induced implies that under ischemic condition, injured cortical neurons have a reduced NGF level and a supply of NGF protein may be helpful to rescue these neurons.

There is evidence for NGF production and NGF-like activity in astrocytes in vitro. The in vivo studies also show that NGF can be expressed in astrocyte-like cells after neostriatal or hippocampal destruction. Giulian reported that after focal cerebral ischemia, some neuronal growth factor could be produced in the damaged rat neocortex, which contained predominantly reactive astrocytes. However, he did not demonstrate what kind of neurotrophic factor was expressed in reactive astrocytes. Our double immunostaining showed that both neurons and reactive astrocytes could express NGF. Because astrocyte-derived NGF can support the survival of neurons, it is possible that reactive astrocytes may play a role in rescuing the ischemic neurons.

In conclusion, our study demonstrated immunohistochemically a temporal profile of NGF protein and its high-affinity trkA receptor in relation to the induction of GFAP and HSP70 after focal cerebral ischemia. The finding that NGF decreases consistently in the ischemic cortex where trkA is induced but can recover in the peri-infarct penumbra area where HSP70 is induced suggested a possible role of NGF/trk receptor system in the mechanism of neuronal injury. Because high-affinity trkA receptor mediates NGF-induced neuronal survival and differentiation, the induction of trkA after focal cerebral ischemia indicated that NGF can act through an autocrine route on the ischemic cortical neurons.

Acknowledgments

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References

NGF and trkA in Focal Cerebral Ischemia


The effects of polypeptide growth factors such as NGF are mediated by high-affinity receptor protein tyrosine kinases (trkA for NGF) expressed on target cells. Growth factor binding to these receptors results in receptor activation, intracellular signal transduction, and specific physiological cellular responses, including cell growth and differentiation. Recent data have demonstrated that a number of growth factors, including NGF, are capable of protecting their target cells from noxious stimuli that induce programmed cell death (apoptosis), such as ionizing radiation, serum starvation, and hypoxia. In most cases, this survival effect has been found to be dependent on activation of phosphatidylinositol 3-kinase and its downstream effectors, and NGF has been shown to activate this signaling pathway to mediate survival of neuronal cells.1,2

In the accompanying article, Lee et al demonstrate that NGF expression is increased early (within 4 hours) after MCA occlusion in cortical neurons and reactive astrocytes distant from the site of cerebral ischemia, including the opposite hemicortex. Meanwhile, expression of the receptor for NGF, trkA, is increased in cells at high risk of injury, those in the peri-infarct penumbra. These findings suggest that a soluble factor is released from the ischemic or infarcted zone to upregulate NGF expression at distant sites. Secreted NGF may then act on increased numbers of trkA receptors in the peri-infarct penumbra to prevent cell death in this region and thereby limit infarct size.

Prior studies have demonstrated that trkA is expressed in a limited subset of cholinergic neurons in the caudate, putamen, and basal forebrain;3 thus, it was not clear that cortical neurons would be capable of responding to increased NGF following cerebral ischemia. However, previous data have shown convincingly that NGF is capable of protecting both cortical and hippocampal neurons from ischemic injury. Although Lee et al did not evaluate patterns of expression of other neurotrophins or their receptors after MCA occlusion, it is possible either that some redundancy exists among the neurotrophins or their receptors after MCA occlusion, it will be important to determine what factor(s) mediates induction of NGF after ischemia. Semkova et al4 have shown that the monoamine oxidase inhibitor selegilene enhances NGF expression and protects central nervous system neurons from ischemic damage. It is possible that selegilene and ischemia utilize similar pathways to induce NGF expression. Further understanding of the mechanisms of NGF induction may lead to the development of drugs that increase endogenous NGF production and have protective effects against cerebral ischemia. Such agents might circumvent the difficulties inherent in administering a polypeptide across the blood-brain barrier.

In summary, further understanding of the basic mechanisms by which cells of the central nervous system survive toxic insults, such as that provided by Lee et al, will likely aid in the development of therapeutic interventions for stroke.

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