Intraventricular Infusion of TrkB-Fc Fusion Protein Promotes Ischemia-Induced Neurogenesis in Adult Rat Dentate Gyrus

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Background and Purpose—We have previously shown that delivery of brain-derived neurotrophic factor (BDNF) through direct intrahippocampal gene transduction with a viral vector suppresses the formation of new dentate granule cells triggered by global forebrain ischemia. Here, we investigated whether inhibition of endogenous BDNF alters ischemia-induced neurogenesis in the dentate gyrus.

Methods—Rats were subjected to 30 minutes of global forebrain ischemia and then received intraventricular infusion of either the BDNF scavenger, TrkB-Fc fusion protein, or control Hu-Fc for 2 weeks. In parallel, all animals were injected intraperitoneally with the mitosis marker 5-bromo-2'-deoxyuridine-5'-monophosphate (BrdU). Animals were killed at 2 or 6 weeks after the ischemic insult, and neurogenesis was then assessed immunocytochemically with epifluorescence or confocal microscopy.

Results—Infusion of TrkB-Fc fusion protein gave rise to elevated numbers of ischemia-generated new neurons, double-labeled with BrdU and the early neuronal marker Hu or the mature neuronal marker NeuN, in the dentate subgranular zone and granule cell layer at 2 and 6 weeks after the insult.

Conclusions—Our findings provide evidence that endogenous BDNF counteracts neuronal differentiation, but not cell proliferation or survival, in ischemia-induced dentate gyrus neurogenesis. (Stroke. 2003;34:2710-2715.)

Key Words: brain-derived neurotrophic factor | cerebral ischemia, global | hippocampus | neurons | stroke | rats

In the dentate gyrus (DG) of the adult brain, new functional granule cells are continuously formed from neural stem cells (NSCs) located in the subgranular zone (SGZ).1–3 Global forebrain ischemia in rodents stimulates the proliferation of NSCs in the SGZ and leads to increased generation of granule cells.4–6 Because ischemic preconditioning, which protects CA1 neurons against subsequent damage, did not prevent ischemia-induced neurogenesis, it is probably not dependent on CA1 neuronal loss.4 In accordance, stroke induced by middle cerebral artery occlusion, which causes striatal and cortical infarction but no cell loss in the hippocampus, also gives rise to a marked increase in DG neurogenesis.7,8

Brain-derived neurotrophic factor (BDNF) plays an important role in neuronal survival and differentiation during embryonic development of the nervous system.9 The functional effect of BDNF is mediated by interaction with its high-affinity receptor, TrkB. BDNF can significantly increase in vitro survival and differentiation of NSCs isolated from embryonic and postnatal hippocampus,10–13 and mutant mice lacking BDNF show increased apoptosis in the SGZ at early postnatal stages.14 Furthermore, intraventricular infusion of BDNF protein15,16 and overexpression of the BDNF gene in the ventricular zone17 in intact, adult rats increase the number of new neurons in the rostral migratory stream and olfactory bulb, striatum, septum, thalamus, and hypothalamus. Global forebrain ischemia has been shown to induce increased endogenous BDNF protein levels in the rat DG.18 This observation raises the possibility that BDNF could be involved in the regulation of ischemia-evoked neurogenesis. However, in contrast to the data obtained in intact animals, we recently found that long-term delivery of BDNF via intrahippocampal transduction of recombinant adeno-associated virus carrying the BDNF gene counteracts neuronal differentiation of cells generated in the DG after global forebrain ischemia in adult rats.19 The main objective of the present study was to explore whether the postischemic increase in endogenous BDNF levels also affects ischemia-induced DG neurogenesis. To scavenge endogenous BDNF, we used intraventricular infusion of TrkB-Fc, which is a fusion protein combining the extracellular binding domain of TrkB and the Fc domain of human IgG.20,21
Materials and Methods

Animals and Experimental Design

Thirty adult, male Wistar rats (Taconic M&B A/S) weighing 280 to 290 g at the time of the ischemic insult were housed under 12-hour light/12-hour dark conditions with ad libitum access to food and water. After fasting overnight with free access to water, all animals were implanted intraventricularly with a cannula connected to an osmotic minipump delivering either TrkB-Fc (n = 10) or control Hu-Fc (n = 20). Immediately thereafter, the rats were subjected to 30 minutes of global forebrain ischemia (n = 10 for TrkB-Fc, n = 11 for Hu-Fc) or sham treatment (n = 9 for Hu-Fc). Starting the next day, all animals received injections of 5-bromo-2'-deoxyuridine-5'-monophosphate (BrdU; twice daily, 50 mg/kg) for 2 weeks. At this time point, 6 rats that had been subjected to the ischemic insult (n = 3 with TrkB-Fc, n = 3 with Hu-Fc) and 3 sham-treated rats were transcardially perfused for immunocytochemistry. The minipumps were removed in the remaining animals, but the rats were not killed until 4 weeks later. In the immunocytochemical analysis, the investigator was blinded to whether TrkB-Fc– or Hu-Fc–containing minipumps were implanted in the individual animals.

Minipump Implantation

Rats were anesthetized with 1% halothane in N2O/O2 (70%/30%), and a cannula connected to an osmotic minipump (Alzet; model L; flow rate, 0.5 μL/h) was then implanted into the right lateral ventricle (0.5 mm caudal to bregma, 1.2 mm lateral from midline, and 3.5 mm ventral from skull with tooth bar −3.2 mm according to the atlas of Paxinos and Watson22). Minipumps were filled with either TrkB-Fc (1 mg/pump; gift from Regeneron Pharmaceuticals) or Hu-Fc (0.34 mg/pump; ICN Biomedicals Inc) in 0.1 mol/L phosphate-buffered saline (PBS; pH 7.0 to 7.4).

Induction of Global Forebrain Ischemia

Animals were anesthetized with 3.5% halothane, intubated, and then artificially ventilated with the halothane concentration lowered to 1.0% to 1.5%. The tail artery was cannulated for blood sampling and pressure recording. Body temperature was measured by a rectally placed thermometer and maintained at 37°C with a temperature controller (CMA/150). The common carotid arteries were isolated, and loose ligatures were placed around them. The jugular vein was cannulated for blood sampling and pressure recording. Ischemia was induced by bilateral occlusion of the common carotid arteries for 30 minutes with hypotension (50 mm Hg). Circulation was restored by removal of the occluding clamps and reinfusion of blood. In the immediate recirculation period, sodium bicarbonate (0.5 mL IV, 50 mg/mL) was given. After regaining spontaneous respiration, the animals were extubated. Sham-operated animals were treated identically except that carotid arteries were not occluded. All animals had physiological parameters within predetermined ranges, and no significant differences were observed between the TrkB-Fc– and Hu-Fc–treated groups subjected to ischemia (data not shown).

Immunocytochemistry

The rats were deeply anesthetized with pentobarbital and transcardially perfused with saline followed by ice-cold 4% paraformaldehyde in 0.1 mol/L phosphate buffer. The brains were removed and postfixed in the same fixative overnight before equilibration in 20% sucrose solution in PBS. All brains were cut into 30-μm-thick coronal sections, which were then stored in a cryoprotective solution at −20°C. For assessing the penetration of TrkB-Fc and Hu-Fc into the brain parenchyma, free-floating sections were stained immunocytochemically with an antibody against the Fc part of human IgG (Hu-IgG; 1:10000; goat polyclonal; Sigma). Briefly, the sections were first rinsed and endogenous peroxidase was quenched in 3% H2O2 and 10% methanol. Subsequently, the sections were incubated with the primary antibody in 2% normal rabbit serum in 0.25% Triton X-100 in potassium and PBS (KPBS) at 4°C overnight. After rinsing, sections were incubated with the biotinylated rabbit anti-goat secondary antibody (1:200; Vector Laboratories) in 2% normal rabbit serum in 0.25% Triton X-100 in KPBS for 1 hour. Then sections were rinsed and incubated in avidin-biotin-peroxidase complex (Elite ABC Kit, Vector Laboratories), and peroxidase was developed by the diaminobenzidine reaction.

For double-labeled fluorescence immunocytochemistry, the following antibodies were used: NeuN (1:100; mouse monoclonal; Chemicon), BrdU (1:100; rat monoclonal; Harlan Sera-Laboratory Ltd), and Hu (1:500; mouse monoclonal; Chemicon). Briefly, free-floating sections were denatured in 1 mol/L hydrochloric acid at 65°C for 30 minutes. After rinsing, the sections were incubated for 36 hours with either BrdU and NeuN or BrdU and Hu antibodies in 5% normal donkey serum and 5% normal horse serum in 0.25% Triton X-100 in KPBS at 4°C. The sections were then rinsed and incubated for 2 hours with 1:200 secondary Cy3-conjugated donkey anti-rat antibody (Jackson ImmunoResearch) and 1:200 secondary biotinylated horse anti-mouse antibody (Vector) in a mixture of 2% normal donkey serum and 2% normal horse serum in 0.25% Triton X-100 in KPBS. After several rinses, sections were incubated for 2 hours with 1:250 Alexa 488–conjugated streptavidin (Molecular Probes) in 0.25% Triton X-100 in KPBS, rinsed, mounted on gelatin-coated slides, and coverslipped with PVA-DABCO mounting medium. When staining for Hu, the streptavidin step was preceded by tyramide amplification procedure (TSA biotin system, NEN).

Microscopical Analysis

Penetration of TrkB-Fc and Hu-Fc into the brain parenchyma was assessed in sections stained with antibody against human IgG using 4× objective in a bright-field microscope. BrdU-positive or BrdU-NeuN and BrdU-Hu double-immunopositive cells were counted using 40× objective in an epifluorescence microscope. Labeled cells within the dentate granule cell layer (GCL) and SGZ ipsilateral to the cannula implantation were counted in 4 coronal sections separated by 300 μm and located −2.8 to −4.2 mm from bregma.

The validity of the double labeling as observed in the epifluorescence microscope was evaluated with a confocal laser scanning microscope (Leica) in 1 randomly chosen section from every other animal. Cells were considered double labeled when BrdU and NeuN or Hu immunoreactivity was colocalized in a minimum of 3 consecutive images in a z series with a 1-μm interval.

Statistical Analysis

All values are given as mean ± SEM. Comparisons between numbers of single- or double-labeled cells and percentages of NeuN- and Hu-positive cells were performed with Student’s unpaired t test. Significance was set at P < 0.05.

Results

The penetration of TrkB-Fc and Hu-Fc into the brain parenchyma from the infusion site in the right lateral ventricle 2 weeks after ischemia closely resembled the previously observed pattern.24 Both TrkB-Fc and Hu-Fc were detected in the rostral part of the hippocampus, in most rats bilaterally, but with a higher staining intensity and wider distribution on the right side. We observed no differences in hippocampal staining pattern between TrkB-Fc– and Hu-Fc–infused animals. The septal region was intensely stained, and both TrkB-Fc and Hu-Fc were detected bilaterally in the dorsomedial striatum. There was also penetration of TrkB-Fc and Hu-Fc into the cerebral cortex, mostly in the cingulate and frontal cortices close to the cannula tract. In rats that had
survived for 4 weeks after removal of the pump, staining was detectable only close to the cannula tract, presumably where the concentration had been the highest. In other areas, immunostaining was absent, indicating that TrkB-Fc and Hu-Fc had been washed away.

In agreement with previous studies, ischemic insult gave rise to a significant increase in the number of BrdU-NeuN double-labeled cells (Figure 1) in the SGZ and GCL of rats infused with Hu-Fc. Elevated numbers of BrdU-NeuN double-positive cells were observed at both 2 and 6 weeks after ischemia (3.6- and 4.1-fold increase, respectively). However, at 6 weeks after the insult, the number of double-labeled cells was higher, probably reflecting the time required for the new neurons to fully express mature neuronal markers such as NeuN (data not shown).

We observed no significant differences in the total number of BrdU-positive cells in the dentate SGZ and GCL between ischemic animals infused with TrkB-Fc or Hu-Fc (Figure 2A). Thus, TrkB-Fc does not influence the number of new cells in these areas at either 2 or 6 weeks after global forebrain ischemia. In addition, the number of BrdU-NeuN double-labeled cells was similar in the 2 groups 2 weeks after ischemia (Figure 2B). However, at 6 weeks after the insult, the animals that had been infused with TrkB-Fc had a significantly higher number of BrdU-NeuN-positive neurons compared with the Hu-Fc–treated rats (Figure 2B). Similarly, whereas there was no difference between the groups at 2 weeks, the proportion of BrdU-positive cells also expressing NeuN immunoreactivity was significantly higher in TrkB-Fc–treated rats (50%) compared with Hu-Fc–treated rats (30%) at 6 weeks (Figure 2C).
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ischemia (Figure 3A and 3B), probably reflecting the earlier division of BrdU-Hu double-labeled cells already at 2 weeks after TrkB-Fc infusion seemed to increase the number and proportion of BrdU-positive cells double labeled withHu (Figure 3B). Interestingly, in contrast to BrdU-NeuN double-labeled cells, BrdU-positive cells generated in these areas is unchanged 2 and 6 weeks after the insult compared with Hu-Fc at 6 weeks after the ischemic insult (Figure 3A). In addition, intraventricular delivery of TrkB-Fc suppresses epileptogenesis,41 similar to what has been observed in heterozygous BDNF knockout mice42 and in transgenic mice overexpressing truncated TrkB receptors and with decreased endogenous BDNF levels.43 In contrast to these data, Croll et al20 reported that TrkB-Fc can potentiate BDNF-induced TrkB phosphorylation. However, this effect was observed only when TrkB-Fc and BDNF were coinjected intracerebrally in equimolar concentrations. Thus, it is highly unlikely that the TrkB-Fc infusion performed in the present study would act by enhancing endogenous BDNF activity.

The present findings provide further support for the hypothesis that BDNF can counteract the neuronal differentiation of new cells generated in the DG after global forebrain ischemia. Local elevation of BDNF levels19 and inhibition of endogenous BDNF, as used here, reduces and increases, respectively, the ischemia-induced neurogenesis. In contrast, cell proliferation and survival are unaffected by these manipulations of BDNF activity. We have suggested19 that BDNF may act by blocking the maturation of the newly generated cells beyond an intermediate developmental stage. In agreement with this interpretation, BDNF-overexpressing cerebellum-derived progenitor cells showed reduced expression of neuronal markers and appeared as round, flattened cells without processes.44 Conversely, the same cells, when genetically manipulated to produce less BDNF by expressing antisense BDNF, exhibited increased expression of neuronal markers and smaller cell bodies, often bearing complex, multiple processes.44

In contrast to the suppressant action of BDNF on the differentiation step in ischemia-induced DG neurogenesis, increased BDNF production evoked by systemic injection of the voltage-dependent sodium channel blocker riluzole has been reported to promote proliferation of DG progenitor cells in the intact rats. This effect could be blocked by intraventricular administration of BDNF antibodies.45 Also, heterozygous BDNF knockout mice with decreased hippocampal BDNF levels have been found to exhibit decreased proliferation of DG progenitors.46 Dietary restriction leading to elevated BDNF levels47 improved the survival of the newly generated DG cells in wild-type and, to a lesser extent, in knockout mice.46 The discrepancies between these data and
our own observations suggest that BDNF has different modulatory actions on basal and ischemia-induced SGZ neurogenesis. Analogously, N-methyl-D-aspartate receptor activation has been reported to reduce basal neurogenesis but to enhance the formation of new DG neurons after both global forebrain ischemia and stroke.7,48

In the other neurogenic area, the SVZ, BDNF seems to promote both basal and insult-induced neurogenesis. Thus, administration of BDNF to the lateral ventricle in intact rats increases the generation of new neurons in the SVZ. Recently, we have observed59 that viral vector-mediated delivery of BDNF to the striatum leads to an increased number of new striatal neurons formed in the SVZ after stroke. The contradictory effects of BDNF on ischemia-induced neurogenesis in the 2 neurogenic areas are in agreement with the idea that the adult SVZ contains multipotential NSCs, whereas neuron-specific progenitors reside in the SGZ.50

We previously hypothesized that the viral vector-mediated long-term delivery of high levels of BDNF to the DG may have acted by downregulating the TrkB receptor.51 Ensuing desensitization of the progenitor cells or their progeny to the elevated endogenous BDNF levels triggered by the cerebral ischemia could therefore explain the subsequent attenuation of neurogenesis.59 Arguing against this possibility is the finding in the present study that TrkB-Fc infusion started at the time of the ischemic insult had an effect opposite that after long-term BDNF delivery.

In conclusion, the results of the present study indicate that intraventricular administration of TrkB-Fc, which most likely leads to decreased activity of endogenous BDNF, increases the formation of new dentate granule cells after 30 minutes of global forebrain ischemia by promoting neuronal differentiation. We have previously demonstrated that intraventricular infusion of TrkB-Fc in rats during 1 week before and 1 week after the same insult aggravates ischemic damage and gives rise to significantly lower number of surviving CA4 pyramidal and neuropeptide Y-immunoreactive dentate hilar neurons.24 Taken together, these studies reveal a remarkable diversity of BDNF function in hippocampal cellular plasticity after global forebrain ischemia.

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References


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