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Glial cell line-derived neurotrophic factor protects against ischemia/hypoxia-induced brain injury in neonatal rat

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Abstract Ischemic/hypoxic brain damage induced in 7-day-old rats was significantly attenuated in a dose-dependent manner by intracerebral injection of glial cell line-derived neurotrophic factor (GDNF; 2 or 4 μg) within 30 min after the insult. Whereas the great majority of the vehicle-treated animals showed massive infarction involving more than 75% of the affected cerebral hemisphere, GDNF injection resulted in a remarkable reduction in both the incidence and severity of the brain damage (incidence ranging from 76% to 93% in controls to 34% to 64% in the 2.0-μg group and 7% to 29% in 4.0-μg group). The induction of immunoreactive 70-kDa heat shock protein (HSP70) in cerebral cortical neurons was also significantly reduced in GDNF-treated animals as compared to controls. The mechanisms responsible for the neuroprotective effects of GDNF remain unknown, although it has been speculated that these may be endogeneous. The higher expression of GDNF and its mRNA in developing brains may be one of the factors responsible for the relative resistance to ischemia of fetal and neonatal as opposed to adult brains. GDNF may possibly act by protecting against oxidative stress or by scavenging free radicals generated during ischemia. The results of our study strongly suggest that GDNF may prove to be an effective and potent protective agent against perinatal ischemic/hypoxic encephalopathy.

Key words GDNF · Neuroprotection · Hypoxia/ischemia · Brain injury · Neonatal rat

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Introduction

Perinatal ischemic/hypoxic encephalopathy, a frequent sequel to perinatal asphyxia, is one of the major causes of childhood neurological disability [37, 38]. No effective means of treatment are as yet available. However, recent progress in our understanding of the pathophysiological factors involved in ischemia/hypoxia-induced neuronal death has suggested new approaches toward prevention of and/or rescue from ischemia-induced brain damage.

Interest has heightened in recent years in the use of neurotrophic factors to provide protection against neurotoxic or axotomy-induced injuries [3, 4, 8, 13, 28, 31, 36, 39]. Neurotrophic factors have been shown to play an important role in the development and maintenance of specific populations of neurons in the central and peripheral nervous system. These include nerve growth factor, brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), insulin-like growth factor, basic fibroblast growth factor (bFGF), transforming growth factor-β3, neurotrophin-3 (NT-3), NT-4/5 and NT-6 [15, 19, 23, 42].

GDNF is a potent peptide neurotrophic factor that has been purified and cloned based on its ability to protect and rescue dopaminergic neurons [26]. Since its discovery, abundant evidence has accumulated to demonstrate its neuroprotective effects against neurotoxic injury to dopaminergic neurons in various experimental paradigms [7, 9, 12–15]. In addition, GDNF has been shown to provide protection for nondopaminergic systems, including motor neurons [16, 29, 41] and peripheral ganglia [5].

A diversity of functional roles for GDNF has been suggested from findings indicating the widespread expression of GDNF [20] and GDNF mRNA [33–35] in neuronal and non-neuronal cells throughout all regions of the developing rat brain. An apparent developmental shift in GDNF expression further suggested that GDNF may be of critical importance at different developmental stages not only for normal brain growth and differentiation but also for the rescue and repair of injured neurons.

The purpose of this study was to examine the effects of GDNF in ischemia/hypoxia-induced brain injury. Although the neuroprotective effects of GDNF against ischemia-induced brain damage in adult rats have been described previously [40], there are significant differences in normal biology and in vulnerability to ischemic/hypoxic injury in the developing brain.

Materials and methods

This study was approved by the Animal Research Committee of Miyazaki Medical college. Pregnant Wistar rats were purchased from Japan Charles River (Shizuoka, Japan).

On postnatal day 7, littermates were assigned to one of three groups: GDNF 2 μg (n = 14), GDNF 4 μg (n = 14) and vehicle [phosphate-buffered saline (PBS) pH 7.40] (n = 14). Each pup was subjected to a modified Levine procedure for producing hypoxic/ischemic injury [25, 30]. Briefly, pups were anesthetized with ether and the left carotid artery was sectioned between double ligatures with 4–0 surgical silk. The pups were allowed to recover for 1–2 h and then exposed to 2 h of hypoxia in a plastic container that was perfused with a mixture of humidified 8% oxygen balanced with nitrogen. The temperature inside the container was kept at 33 °C, the usual temperature to which rat pups are exposed when huddling with the mother [27].

Within thirty minutes after removal from the hypoxic chamber, pups received 5-μl intracerebral injections of either vehicle (PBS) or 2 or 4 μg GDNF dissolved in PBS. Injection was performed with a 10-μl Hamilton syringe and a 27-gauge needle. The injection site was 2.0 mm anterior and 1.5 mm lateral to the lambda, and 1.0 mm deep to the skull surface. Recombinant rat GDNF was purchased from R&D Systems (512-GF, Minneapolis, MN).

One week after the ischemic/hypoxic insult, animals were anesthetized and killed, and the brains removed and fixed in ethanol-acetic acid (19 : 1) for 24 h. Coronal brain slices cut 2 and 6 mm anterior to the interaural line and containing the striatum and dorsal hippocampus, respectively, were embedded in paraffin. Sections for light microscopy were cut at 5–8 μm and stained with hematoxylin and eosin. Adjacent sections were processed for glial fibrillary acidic protein (GFAP) immunocytochemistry. Selected samples were also processed for immunocytochemical staining for terminal deoxynucleotidyl transferase-catalyzed dUTP-biotin nick labeling (TUNEL).

In another series, 7-day-old rats were used to assess expression of immunoreactive 70-kDa heat shock protein (HSP70) in brains of control (vehicle, n = 5) and experimental (4.0 μg GDNF, n = 5) groups 24 h after induction of ischemic/hypoxic brain damage.

Ischemic/hypoxic damage induced in the parietal and frontal cortices, CA1, CA3 and the dentate gyrus of the hippocampus, the striatum and the thalamus was evaluated microscopically in coded sections by different investigators, according to an arbitrary scale of severity, as described previously [30]: none, no damage; mild, 25% or less of the surface area in a single section; moderate, more than 25% but less than 50%; and severe, more than 50%.

Camera lucida drawings of each brain section from every animal were scanned using a Hewlett-Packard Scanner, and the scanned images were saved in a picture file and imported into an NIH-image software program. The damaged area in each section was outlined planimetrically and measured by counting the number of enclosed pixels and expressing the count as a percentage of total hemispheric area.

Nonparametric statistics were used for statistical analyses since data were not normally distributed. The Mann-Whitney rank sum test was used to compare groups.

Results

No animals were lost during surgery, hypoxic stress or recovery phase. No significant differences were observed in weight gain among the three groups of experimental animals (Table 1). As shown in Table 2, the great majority of the vehicle-treated animals demonstrated massive infarction involving more than 75% of the cerebral cortex of the injured hemisphere. Complete necrosis with tissue breakdown and cavitation was the common outcome in both frontal and parietal lobes (Fig. 1). Coagulated necrotic cellular debris mixed with scattered macrophages filled the cavities. Often, eosinophilic ischemic neurons were identified singly or in clusters within the cerebral cortical plate adjacent to necrotic areas. Pyramidal neurons in the CA1 and CA3 regions of the hippocampus showed various degrees of ischemic neuronal damage. Granular neurons of

Table 1 Weight gains in experimental groups (*GDNF* glial cell line-derived neurotrophic factor)

Postnatal day	Vehicle mean ± SEM	2 μg GDNF	4 μg GDNF
7	13.8 ± 0.3	13.6 ± 0.3	14.0 ± 0.4
14	21.6 ± 0.7	23.9 ± 1.1	23.2 ± 0.7

Table 2 Number of animals with histological grading of damage following ischemic/hypoxic insults: none, no damage; mild, 25% or less of surface area on a single section; moderate, more than 25% but less than 50%; severe, more than 50%. The incidence of damage was significantly decreased ($P < 0.05$) in both the 2 μg and 4 μg groups as compared to controls

	None	Mild	Moderate	Severe
Vehicle (n = 14)				
Parietal cortex	2	1	2	9
Frontal cortex	2	0	3	9
Hippocampus CA1	2	0	2	10
Hippocampus CA3	1	1	1	11
Hippocampus Dentate	3	2	2	7
Thalamus	2	1	4	7
Striatum	1	2	3	8
2 μg GDNF (n = 14)				
Parietal cortex	7	1	2	4
Frontal cortex	6	1	3	4
Hippocampus CA1	6	2	1	5
Hippocampus CA3	5	0	1	8
Hippocampus Dentate	10	2	2	0
Thalamus	7	4	3	0
Striatum	7	0	1	6
4 μg GDNF (n = 14)				
Parietal cortex	11	0	1	2
Frontal cortex	12	2	0	0
Hippocampus CA1	11	0	2	1
Hippocampus CA3	10	0	1	3
Hippocampus Dentate	13	1	0	0
Thalamus	12	2	0	0
Striatum	11	0	2	1

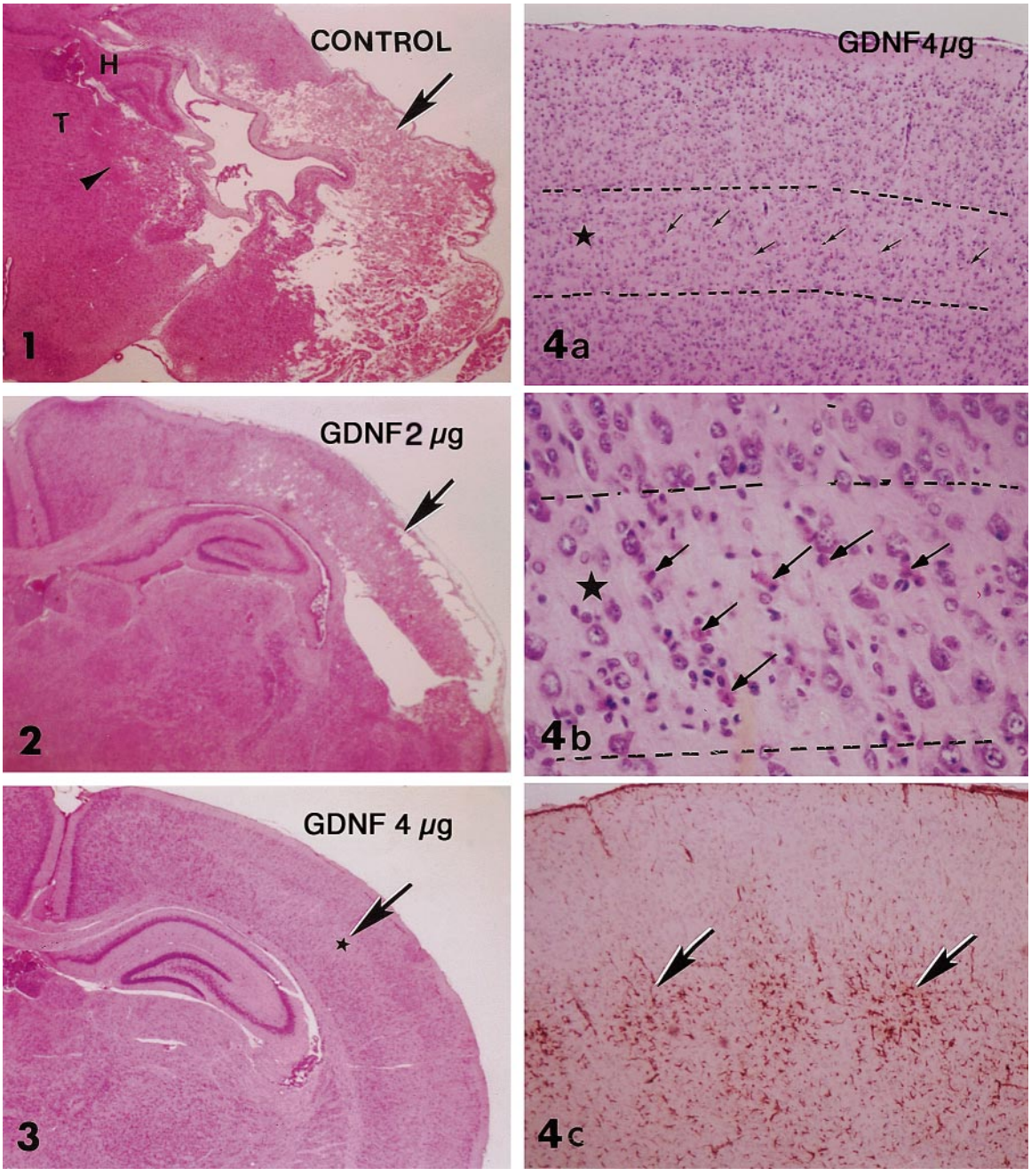

Fig. 1 Photomicrograph showing massive infarction *(arrow)* in the parietal cortex of a control animal. An *arrowhead* points to ischemic necrosis involving the thalamus *(T)*. The hippocampus *(H)* also shows ischemic necrosis. H&E, × 12.5

Fig. 2 Photomicrograph showing infarction *(arrow)* in the parietal lobe of animal treated with 2 μg GDNF. Note the significant reduction in the size of infarction as compared to that in the control (*GDNF* glial cell line-derived neurotrophic factor). H&E, × 12.5

Fig. 3 Photomicrograph showing what appeared to be relatively normal cerebral cortex in the 4-μg GDNF group. However, closer examination revealed the presence of scattered eosinophilic neurons in the deeper layers of the cortex, as indicated by an *asterisk*. H&E, × 12.5

Fig. 4 **a** High magnification of the area indicated by the *asterisk* in Fig. 3, showing the presence of scattered ischemic neurons *(small arrows)* in the deeper layers of the cortex as outlined; 4-μg GDNF group. **b** Higher magnification of **a**, showing the presence of ischemic neurons *(arrows)* in the deeper layers of the cortex; 4-μg GDNF group. **c** Photomicrograph of section adjacent to **a**, showing clusters of GFAP-positive reactive astroglial cells occupying the deeper layers of the cerebral cortical plate, indicating the presence of ischemic damage; 4-μg GDNF group, immunoperoxidase staining. **a**, **c** × 20; **b** × 100

the dentate gyrus also demonstrated diffuse and focal neuronal degeneration. The striatum and thalamus were the seat of focal infarcts mostly confined dorsally and laterally (Fig. 1).

Histologically, degenerating neurons were characterized by shrinkage and eosinophilia of the cytoplasm and pyknosis of the nuclei. In addition to karyorrhexis, fragmentation of the nuclear chromatin suggestive of apoptosis was frequently observed among degenerating neuronal nuclei. TUNEL staining was positive in many. A significant reduction in the number of cells showing TUNEL staining-positive nuclei was noted among GDNF-treated animals (not shown). Prominent GFAP immunoreactivity indicative of astroglial reactivity was observed in damaged areas within the cortex and subcortical regions (see Fig. 4c).

GDNF injection resulted in a marked reduction in both the incidence and severity of ischemic/hypoxic brain damage in a dose-dependent manner (Table 2). In the vehicle-treated group, the incidence of ischemic/hypoxic damage in each selected brain region ranged from 76% to 93%, whereas the incidence decreased to 34–64% in the 2-μg group ($P < 0.05$) and, even more dramatically, to 7–29% in the 4-μg group.

Although infarcts varying from mild to severe grades were still present in the 2-μg group (Fig. 2), there was a significant reduction in the incidence of severe damage and a striking increase in the number of animals showing no apparent histological evidence of damage, as compared to those in the vehicle-treated group (Table 2). A commonly observed feature in the 4-μg group was what appeared to be relatively normal histological appearance of the cerebral cortex (Fig. 3) except for the presence of a small number of scattered eosinophilic neurons in the 4th and 5th layers of the cerebral cortex, which upon closer examination showed ischemic/hypoxic damage (Figs. 4a, 4b). In many animals, clusters of GFAP-positive reactive astrocytes in the neural parenchyma indicated the presence of ischemic/hypoxic injury (Fig. 4c).

The highly significant and dose-dependent reduction of infarct size in GDNF-treated animals as compared to that of the vehicle-treated groups is demonstrated in Fig. 7.

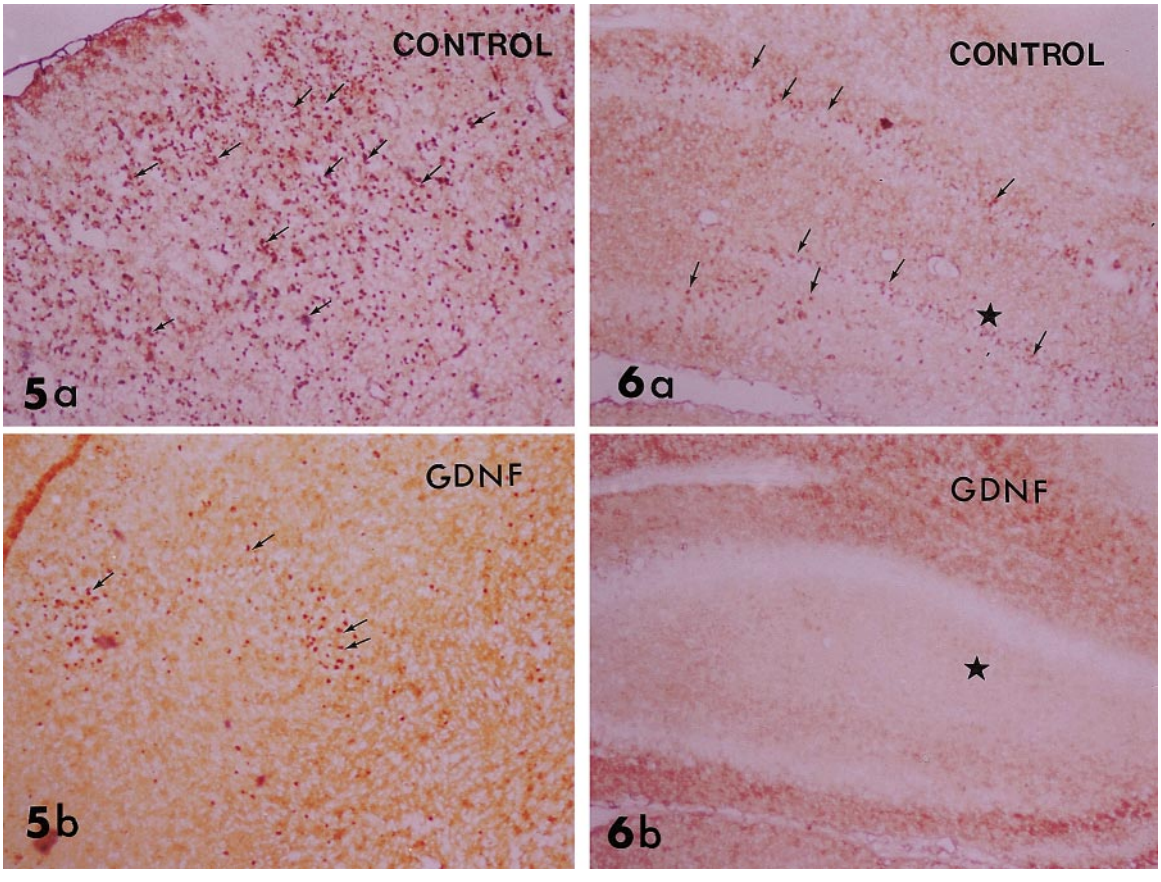

Fig. 5 **a** HSP70 expression among cortical neurons in infarcted cortex of the control group. Note discretely immunoreactive cortical neurons *(arrows)* diffusely scattered in the cortical plate. **b** HSP70 expression among cortical neurons in the GDNF-treated group. Note the highly significant reduction in HSP expression *(arrows)* (*HSP70* 70-kDa heat shock protein). **a**, **b** Immunoperoxidase staining, × 20

Fig. 6 **a** HSP70 expression in the hippocampus of control group. The *asterisk* is placed in the hilar region of the hippocampus. Note HSP70 immunoreactivity is discrete among pyramidal neurons. **b** HSP70 expression in the hippocampus of a GDNF-treated animal, showing no immunoreactivity. The *asterisk* is placed in the hilar region. **a**, **b** Immunoperoxidase staining, × 20

In the vehicle-treated group the damaged area occupied 41.3 ± 2.8% of the total hemispheric area on that side. The percentage decreased highly significantly to 13.4 ± 3.6% ($P < 0.05$) and 1.2 ± 0.8% ($P < 0.05$) in the GDNF 2-μg and GDNF 4-μg groups, respectively.

Immunoreactivity for HSP70 was prominently and diffusely noted among cortical as well as hippocampal neurons in all of the vehicle-treated animals (Figs. 5a, 6a), whereas that in GDNF-treated animals was limited to cerebral cortical neurons adjacent to injection site (Fig. 5b). Hippocampal neurons in GDNF-treated animals showed no immunoreactivity for HSP70 at 24 h after the ischemic/hypoxic insults (Fig. 6b).

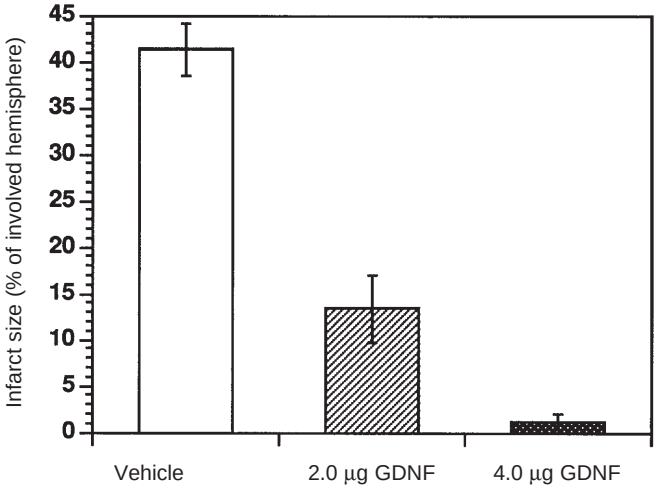

Fig. 7 Comparison of infarct size. Camera Lucida tracings of each brain section from every animal were scanned. The damaged area was outlined planimetrically in each section, and measured by counting the number of enclosed pixels. The data are expressed as a percentage of the total area of the involved hemisphere

Discussion

The results of our study clearly show that GDNF provides remarkable protection against ischemia/hypoxia-induced brain damage in neonatal rats in a dose-dependent manner. Such protection was obtained by intracerebral injection of GDNF within 30 min after the insult. Whereas the great majority of animals in the vehicle (control) group demonstrated the presence of massive infarction involving a major portion of the affected hemisphere, GDNF-treated animals showed a highly significant reduction in both the incidence and severity of damage. Animals treated with 4.0 μg GDNF often showed what appeared to be normal histological patterning of the cerebral cortex except for the presence, on closer examination, of scattered eosinophilic neurons within the deeper layers of the cortex associated with clustering of GFAP-positive astroglial cells (Fig. 4).

The mechanisms responsible for the neuroprotective effects of GDNF remain to be elucidated. It has been speculated that the greater expression of GDNF [20] and its mRNA [34, 35] in developing brains may be one of the factors responsible for the relative resistance of fetal and neonatal brains as compared to adult brains [10, 40]. Up-regulation of GDNF mRNA in rat brains occurs following various types of injury [17, 18]. Therefore, GDNF may exert its effects through endogeneous neuroprotective mechanisms. Undoubtedly, determination of the localization and expression of GDNF and its receptor components [6, 21, 22] is critical not only for an understanding of the functions of GDNF in vivo but also for identification of cells and tissues that preferentially respond to GDNF and its related family of neurotrophic factors.

It has been suggested that GDNF may provide protection against oxidative stress [15]. Nitric oxide (NO) levels rise during ischemia and reperfusion [32], and inhibition of NO synthesis reduces ischemia/hypoxia-induced brain damage [43]. NO also mediates glutamatergic neurotoxicity during brain ischemia, and administration of *N*-methyl-D-aspartate antagonists reduces both NO synthase activity and the severity of infarction [11]. Apparently, GDNF administration completely blocks the increase in NO that accompanies middle cerebral artery occlusion in adult rats [40]. Therefore, GDNF may have the capacity to scavenge free radicals generated during ischemic injury in the brain.

In contrast to prominent and diffuse expression of HSP70 in cortical and hippocampal neurons in the affected cerebral hemisphere of the vehicle-treated animals, GDNF-treated animals demonstrated only limited HSP70 expression among neurons near the needle tract and no HSP70 immunoreactivity among hippocampal neurons. Since HSP is induced, in part, by denatured proteins produced in response to stressful conditions including ischemia, it is possible that GDNF may exert its effects by modulating degenerative processes induced by ischemic/hypoxic damage.

Marked age-dependent neuroprotection against hypoxic/ischemic brain damage has also been observed with BDNF [8]. Although the mechanisms remain unknown, it has been suggested that BDNF may act through intracellular mediators (such as bcl-2) that play a role in apoptotic cell death [2]. bFGF is another neurotrophic factor that has been shown to protect against ischemic brain damage and, apparently, bFGF increases the activities of free-radical-scavenging enzymes, including superoxide dismutase and glutathione reductase [4].

As stated earlier, GDNF pretreatment has also been reported to diminish the volume and extent of infarction significantly following middle cerebral artery occlusion in adult rats [40]. Significant amelioration of cerebral edema induced by transient middle cerebral artery occlusion has also been reported following topical application of GDNF to the brain surface in adult rats [1]. These findings, taken together, suggest that GDNF may prove to be an effective and potent protective agent against perinatal ischemic/hypoxic encephalopathy.

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References

1. Abe K, Hayashi T, Itoyama Y (1997) Amelioration of brain edema by topical application of glial cell line-derived neurotrophic factor in reperfused rat brain. Neurosci Lett 231: 37–40
2. Allsopp TE, Kiselev S, Wyatt S, Davies AM (1995) Role of bcl-2 in the brain-derived neurotrophic factor survival response. Eur J Neurosci 7: 1266–1272
3. Arenas E, Trupp M, Åkerud P, Ibáñez CF (1995) GDNF prevents degeneration and promotes the phenotype of brain noradrenergic neurons in vivo. Neuron 15: 1465–1473
4. Ay H, Ay I, Koroshetz WJ, Finklestein SP (1999) Potential usefulness of basic fibroblast growth factor as a treatment for stroke. Cerebrovasc Dis 9: 131–135
5. Buj-Bello A, Buchman VL, Horton A, Rosenthal A, Davies AM (1995) GDNF is an age-specific survival factor for sensory and autonomic neurons. Neuron 15: 821–828
6. Buj-Bello A, Adu J, Piñón LGP, Horton A, Thompson J, Rosenthal A, Chinchetru M, Buchman VL, Davies AM (1997) Neurturin responsiveness requires a GPI-linked receptor and the Ret receptor tyrosine kinase. Nature 387: 721–724
7. Cass WA, Manning MW (1999) GDNF protection against 6-OHDA-induced reductions in potassium-evoked overflow of striatal dopamine. J Neurosci 19: 1416–1423
8. Cheng Y, Gidday JM, Yan Q, Shah AR, Holtzman DM (1997) Marked age-dependent neuroprotection by brain-derived neurotrophic factor against neonatal hypoxic-ischemic brain injury. Ann Neurol 41: 521–529
9. Date I, Aoi M, Tomita S, Collins F, Ohmoto T (1998) GDNF administration induces recovery of the nigrostriatal dopaminergic system both in young and aged parkinsonian mice. NeuroReport 9: 2365–2369
10. Du C, Hu R, Csernansky CA, Hsu CY, Choi DW (1996) Very delayed infarction after mild focal cerebral ischemia: a role for apoptosis? J Cereb Blood Flow Metab 16: 195–201
11. Earley B, Canney M, Clune B, Caldwell M, Leonard BE, Junien JL (1996) The effects of MK-801, ifenprodil, JO 1784 and JO 1997 on PK 11195 receptor binding nitric oxide synthase (NO synthase) activity and infarct volume in a mouse model of focal cerebral ischaemia. Neurochem Int 28: 509–521
12. Feng L, Wang C-Y, Jiang H, Oho C, Mizuno K, Dugich-Djordjevic M, Lu B (1999) Differential effects of GDNF and BDNF on cultured ventral mesencephalic neurons. Mol Brain Res 66: 62–70
13. Gash DM, Zhang Z, Ovadia A, Cass WA, Yi A, Simmerman L, Russell D, Martin D, Lapchak PA, Collins F, Hoffer BJ, Gerhardt GA (1996) Functional recovery in parkinsonian monkeys treated with GDNF. Nature 380: 252–255
14. Gerhardt GA, Cass WA, Huettl P, Brock S, Zhang Z, Gash DM (1999) GDNF improves dopamine function in the substantia nigra but not the putamen of unilateral MPTP-lesioned rhesus monkeys. Brain Res 81:: 163–171
15. Gong L, Wyatt RJ, Baker I, Masserano JM (1999) Brain-derived and glial cell line-derived neurotrophic factors protect a catecholaminergic cell line from dopamine-induced cell death. Neurosci Lett 263: 153–156
16. Henderson CE, Phillips HS, Pollock RA, Davies AM, Lemeulle C, Armanini MP, Simpson LC, Moffet B, Vandien RA, Kolietsos VE, Rosenthal A (1994) GDNF: a potent survival factor for motoneurons present in peripheral nerve and muscle. Science 266: 1062–1064
17. Ho A, Gore AC, Weickert CS, Blum M (1995) Glutamate regulation of GDNF gene expression in the striatum and primary striatal astrocytes. NeuroReport 6: 1326–1330
18. Humpel C, Hoffer B, Stromberg I, Becktesh S, Collins F, Olson L (1994) Neurons of the hippocampal formation express glial cell line-derived neurotrophic factor messenger RNA in response to kainate-induced excitation. Neuroscience 59: 791–795
19. Hyman C, Juhasz M, Jackson C, Wright P, Ip NY, Lindsay RM (1994) Overlapping and distinct actions of the neurotrophins BDNF, NT-3, and NT-4/5 on cultured dopaminergic and GABAergic neurons of the ventral mesencephalon. J Neurosci 14: 335–347
20. Ikeda T, Xia XY, Xiy Y, Ikenoue T, Choi BH (1999) Expression of glial cell line-derived neurotrophic factor in the brain and cerebrospinal fluid of the developing rat. Int J Dev Neurosci (in press)
21. Kokaia Z, Airaksinen MS, Nanobashvili A, Larsson E, Jujamaki E, Lindavall I. Saarma M (1999) GDNF family ligands and receptors are differentially regulated after brain insults in the rat. Eur J Neurosci 11: 1202–1216
22. Kotzbauer PT, Lampe PA, Heuckeroth RO, Golden JP, Creedon DJ, Johnson EM Jr, Milbrandt J (1996) Neurturin, a relative of glial cell line-derived neurotrophic factor. Nature 384: 467–470
23. Krieglstein K, Maysinger D, Unsicker K (1996) The survival response of mesencephalic dopaminergic neurons to the neurotrophins BDNF and NT-4 requires priming with serum: comparison with members of the TGF-beta superfamily and characterization of the serum-free culture system. J Neural Transm Suppl 47: 247–258
24. Lapchak PA, Gash DM, Collins F, Hilt D, Miller PJ, Araujo DM (1997) Pharmacological activities of glial cell line-derived neurotrophic factor (GDNF): preclinical development and application to the treatment of Parkinson's disease. Exp Neurol 145: 309–321
25. Levine S (1960) Anoxic-ischemic encephalopathy in rats. Am J Pathol 36: 1–17
26. Lin L-F H, Doherty DH, Lile JD, Bektesh S, Collins F (1993) GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. Science 260: 1130–1132
27. Mortola JP, Dotta A (1992) Effects of hypoxia and ambient temperature on gasseous metabolism of newborn rats. Am J Physiol 263: R267–272
28. Olson L (1997) The coming of age of the GDNF family and its receptors: gene delivery in a rat Parkinson model may have clinical implications. Trends Neurosci 20: 277–279
29. Oppenheim RW, Houenou LJ, Johnson JE, Lin LH, Lin L, Lo AC, Newsome AL, Prevette DM, Wang S (1995) Developing motor neurons rescued from programmed and axotomy-induced cell death by GDNF. Nature 373: 344–346
30. Ota A, Ikeda T, Ikenoue T, Toshimori K (1997) Sequence of neuronal responses assessed by immunohistochemistry in the newborn rat brain after hypoxia-ischemia. Am J Obstet Gynecol 177: 519–526
31. Rosenblad C, Kirik D, Devaux B, Moffat B, Phillips HS, Björklund A (1999) Protection and regeneration of nigral dopaminergic neurons by neurturin or GDNF in a partial lesion model of Parkinson's disease after administration into the striatum or the lateral ventricle. Eur J Neurosci 11: 1554–1566
32. Sato S, Tominaga T, Ohnish T, Ohnishi ST (1994) Electron paramagnetic resonance study on nitric oxide production during brain focal ischemia and reperfusion in the rat. Brain Res 647: 91–96
33. Schaar DG, Sieber B-A, Dreyfus CF, Black IB (1993) Regional and cell-specific expression of GDNF in rat brain. Exp Neurol 124: 368–371
34. Springer JE, Mu X, Bergmann LW, Trojanowski JQ (1994) Expression of GDNF mRNA in rat and human nervous tissue. Exp Neurol 127: 167–170
35. Strömberg I, Björklund L, Johansson M, Tomac A, Collins F, Olson L, Hoffer B, Humpel C (1993) Glial cell line-derived neurotrophic factor is expressed in the developing but not adult striatum and stimulates developing dopamine neurons in vivo. Exp Neurol 124: 401–412
36. Tomac A, Lindqvist E, Lin LH, Ogren SO, Young D, Hoffer BJ, Olson L (1995) Protection and repair of the nigrostriatal dopaminergic system by GDNF in vivo. Nature 373: 335–339

37. Vanucci RC (1990) Experimental biology of cerebral hypoxia-ischemia: relation to perinatal brain damage. Pediatr Res 27: 317–326
38. Volpe JJ (1993) Neurology of the newborn, 3rd edn. Saunders, Philadelphia
39. Walton KM (1999) GDNF. A novel factor with therapeutic potential for neurodegenerative disorders. Mol Neurobiol 19: 43–59
40. Wang Y, Lin S-Z, Chiou A-L, Williams LR, Hoffer BJ (1997) Glial cell line-derived neurotrophic factor protects against ischemia-induced injury in the cerebral cortex. J Neurosci 17: 4341–4348
41. Yan Q, Matheson C, Lopez OT (1995) In vivo neurotrophic effects of GDNF on neonatal and adult facial motor neurons. Nature 373: 341–344
42. Zawada WM, Zastrow DJ, Clarkson ED, Adams FS, Bell KP, Freed CR (1998) Growth factors improve immediate survival of embryonic dopamine neurons after transplantation into rats. Brain Res 786: 96–103
43. Zhang ZG, Reif D, MacDonald J, Tang WX, Kamp DK, Gentile RJ, Shakespeare WC, Murray RJ, Chopp M (1996) ARI 17477, a potent and selective neuronal NOS inhibitor decreases infarct volume after transient middle cerebral artery occlusion in rats. J Cereb Blood flow Metab 16: 599–604