

INCREASED EXPRESSION OF GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR IN RAT BRAIN AFTER TRAUMATIC BRAIN INJURY

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Abstract- Glial cell line-derived neurotrophic factor (GDNF) plays important roles not only for the differentiation of neurons during normal development but also for the survival and recovery of many populations of mature neurons. The effect of traumatic brain injury (TBI) on the expression of GDNF is currently unknown. To determine if there is alteration in GDNF after TBI we examined the effect of controlled cortical impact (CCI) injury on GDNF protein levels at 6 hours, 1 day, 1 week, and 4 weeks following injury by utilizing a commercially available antibody specific to GDNF. Rats were anesthetized and surgically prepared for CCI injury (4 m/sec, 2.7 mm) and sham surgery. Injured and sham animals (n=6 per group) were sacrificed at 6 hours, 1 day, 1 week, and 4 weeks and perfused with 4% paraformaldehyde. Coronal sections (35 mm thick) were cut through the hippocampus. An increased expression of GDNF protein was observed by immunohistochemistry in the dentate gyrus of hippocampus and the cortex in injured rats compared to sham controls. The increased expression of GDNF was more evidently observed in the ipsilateral dentate gyrus and the area around the contusion in the cortex. In the cortex, GDNF immunoreactivity appeared greatest in cells with glial morphology but in the hippocampus, GDNF immunoreactivity was greatest in neuronal-like cells. These changes were observed at 1 day, 1 and 4 weeks postinjury. We speculate that the up-regulation of the GDNF protein may reflect its neurotrophic and neuroprotective effect on dopaminergic system responding to the TBI insult.

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Key words: Glial cell line-derived neurotrophic factor, rat, traumatic brain injury, immunohistochemistry, cortex, dentate gyrus

INTRODUCTION

Glial cell line-derived neurotrophic factor (GDNF) is synthesized as a precursor and secreted as a mature protein of 134 amino acids. It contains the seven conserved and typically spaced cysteine residues found in all members of the transforming

growth factor- β (TGF- β) superfamily but shares less than 20% identity with any family member. The apparent mass of the monomer is about 16 kDa after deglycosylation. The amino acid sequences of rat and human GDNF are 93% identical. Studies aimed at identifying the sites of GDNF mRNA production have been carried out by a number of research groups. GDNF plays important roles not only for the differentiation of neurons during normal development but also for survival and recovery of many populations of mature neurons. GDNF is a potent and relatively specific neurotrophic factor for dopaminergic neurons. It has been reported that

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GDNF after traumatic brain injury

GDNF has protective effects on various injuries of central and peripheral nervous systems *in vitro* and *in vivo* (1). A variety of studies have provided evidence for restorative or neuroprotective effects of GDNF on dopaminergic neurons following injury to the nigrostriatal pathway and then shown to rescue these neurons in animal models of Parkinson's disease (2-5). Recent evidence indicates that exogenous administration of GDNF can also rescue the septal cholinergic cells from death following fimbria/fornix transections and significantly reduce the infarct size and brain edema after ischemic brain injury (6). However, the effect of traumatic brain injury (TBI) on the expression of GDNF is currently unknown. To determine if there is alteration in expression of GDNF after TBI, we examined the effect of controlled cortical impact (CCI) injury on GDNF protein levels at 6 hours, 1 day, 1 week, and 4 weeks following injury by utilizing a commercially available antibody specific to GDNF.

MATERIALS AND METHODS

All surgical procedures were conducted and animals were cared for according to the animal welfare guidelines set forth in the guide for the Care and Use of Laboratory Animals (Publication 85-23, United States Department of Health and Human Services, 1985).

Six adult male Sprague-Dawley rats weighing 250 to 300 g were anesthetized with isoflurane (induction dose 5%, maintenance dose 1.25%) in a 2:1 N₂O/O₂ mixture. Each animal was placed in a stereotactic head frame and a 5-cm scalp and neck incision was made vertically. The scalp and temporalis muscles were reflected and a 6-mm craniectomy was performed between the coronal and lambdoid sutures 1 cm lateral to the midline. The dura was kept intact and care was taken to avoid injury to the vasculature. The TBI was induced at a velocity of 4 m/second, resulting in a 2.8 mm cortical tissue deformation, by using the CCI device. Six sham-operated control rats were subjected to the same surgical procedure, including craniectomy, but received no cortical impact. All animals were randomly subjected to either a CCI injury or sham surgery (7, 8). Body

temperature was monitored with rectal probe. Reflexes including flexion in right and left lower extremity, tail pinch, pinna, corneal, and righting were checked in sham and injured rats and charted in seconds. After recovery from anesthesia, the rats were returned to their cages, where they had free access to food and water.

The controlled cortical impact injury device consists of a small (1.975 cm) bore, double-acting, stroke-constrained, pneumatic cylinder with a 5.0 cm stroke. The cylinder is rigidly mounted in a vertical position on a crossbar, which can be precisely adjusted in the vertical axis. The lower rod end has an impactor tip (6 mm diameter) attached (*i.e.*, part of the shaft that comes into contact with the exposed dura mater). The upper rod end is attached to the transducer core of a linear velocity displacement transducer (LVDT). The velocity of the impactor shaft is controlled by gas pressure. Impact velocity is measured directly by the LVDT (Schacvitz Model 500 HR) that produces an analog signal that is recorded by a PC-based data acquisition system (R.C. Electronics) for analysis of time/displacement parameters of the impactor (Fig. 1).

Animals were deeply anesthetized with pentobarbital (Nembutal, 80-100mg/kg; Abbott laboratories, North Chicago, IL) at 6 hours, 1 day, 1 week, and 4 weeks after injured (n=6) and sham injured operation (n=6). The rats were transcardially perfused and coronal sections were cut (35-40 mm thickness) on a cryostat. Immunohistochemistry for GDNF was conducted in 24-well culture plates by free-floating technique. Sections were incubated with primary antibody, rabbit anti-GDNF antibody (1:60;

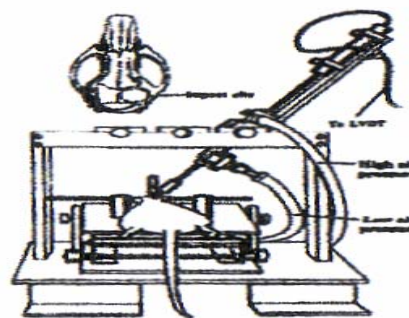


Fig. 1. Controlled cortical impact device.

Santa Cruz Biotechnology) with mouse anti-NeuN (1:600) or anti-glia fibrillary acidic protein (GFAP) (1:600) monoclonal antibodies (Chemicon International) at 4°C for 16-24 hours. Goat anti-rabbit IgG conjugated with Cy3 fluorescence dye (1:300; Jackson Immunoresearch laboratories) were used for GDNF and NeuN or GDNF and GFAP double labeling study, respectively. At least six sections of brain tissue through dorsal hippocampus for each animal were processed for immunoactivity. The fluorescence images of immunohistochemistry were captured by a laser confocal microscope (Carl Zeiss).

RESULTS

An increased expression of GDNF protein was observed by immunohistochemistry in the dentate gyrus and the cortex in injured rats compared to sham controls starting at one day after TBI insult. The increased expression of GDNF was more evidently observed in the ipsilateral dentate gyrus and the area around the contusion in the cortex.

Double labeling immunofluorescence studies revealed that GDNF are expressed by neurons but not by astrocytes although overexpression of GFAP was noted after TBI (Fig. 2).

DISCUSSION

An increased expression of GDNF was observed in the ipsilateral dentate gyrus and the cortex in injured rats starting at one day after TBI. Study of Wei *et al.* revealed expression of GDNF mRNA and protein was first increased as early as 2 h after ischemia-reperfusion in peri-infarct cerebral cortex and striatum; it then declined and showed a second increase at 72 h. Double staining confirmed that the earlier peak of GDNF expression was of neuronal origin and the later peak of glial origin (9).

Miyazaki *et al.* showed transient forebrain ischemia induced GDNF mRNA expression in the hippocampus from 3 h to 3 days after the ischemic episode, with peak expression at 6 h. The GDNF mRNA increase in the cerebral cortex was similar to that in the hippocampus, whereas no increase in GDNF mRNA was observed in the striatum and brainstem (10).

Koo and Choi revealed GDNF expression in developing human fetal brains. At 7-8 weeks, strong immunoreactivity was noted within radial glial processes, glia limitans and choroid plexus of the telencephalic vesicle. By 10 weeks, ependymal cells, primitive matrix cells and early developing cortical plate neurons showed positive staining. By 15-16

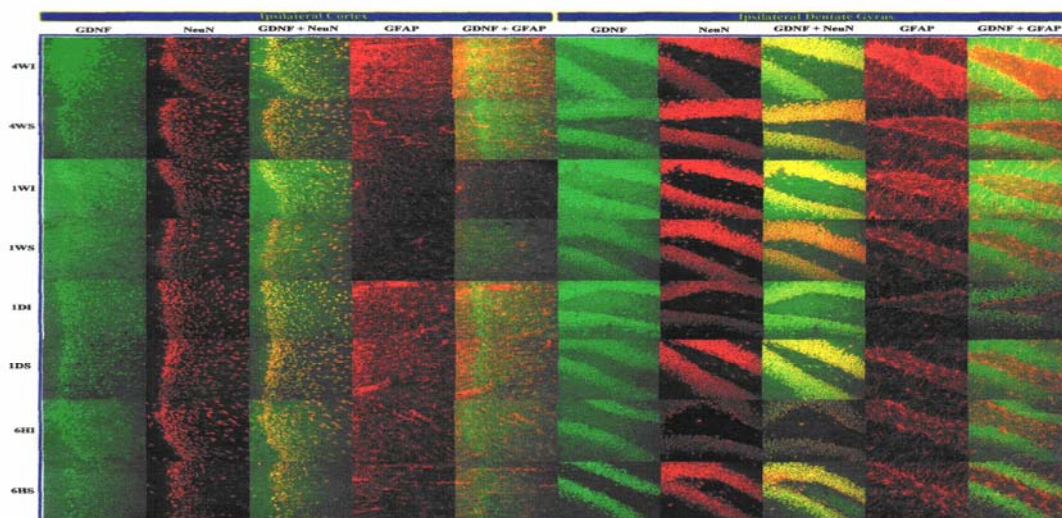


Fig. 2. An increased expression of glial cell line-derived neurotrophic factor (GDNF) was observed in the ipsilateral dentate gyrus and the cortex in injured rats compared to sham controls starting at one day after traumatic brain injury. GDNF is expressed by neurons but not by astrocytes although over-expression of glial fibrillary acidic protein was noted.

weeks, migrating neurons in the subventricular and intermediate zones and in the cortical plate were strongly positive for GDNF. The glia limitans of the cerebral cortex and subependymal astrocytes remained positive at this time.

As fetal age increased, GDNF expression shifted to neurons and glial cells in the deeper structures of the brain. The most prominent GDNF staining was observed in the cytoplasm and dendrites of Purkinje cells of the cerebellum by 25 weeks and thereafter. Pyramidal neurons of the CA1 region and granule cells of the dentate fascia of the hippocampus, neurons of the entorhinal cortex, and scattered neurons within the brain stem, medulla and spinal cord all showed strong GDNF staining by 25-35 weeks (11).

Kim *et al.* showed that compared with control rats infused with artificial cerebrospinal fluid, GDNF infusion significantly decreased the TBI-induced neuronal loss in both the CA2 and CA3 regions (12). There was no difference in the number of GF AP-positive astroglial cells in the GDNF-infused rats in the TBI and sham-operated groups compared with the respective vehicle-treated groups. The up-regulation of the GDNF protein may reflect its neurotrophic and neuroprotective effect on dopaminergic system responding to the TBI insult.

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