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SCIENCE DIRECT

Brain Research 1052 (2005) 147 – 155

BRAIN RESEARCH

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Research Report

Increased growth factor expression and cell proliferation after contusive spinal cord injury

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Accepted 27 May 2005
Available online 7 July 2005

Abstract

The damage caused by traumatic central nervous system (CNS) injury can be divided into two phases: primary and secondary. The initial injury destroys many of the local neurons and glia and triggers secondary mechanisms that result in further cell loss. Approximately 50% of the astrocytes and oligodendrocytes in the spared white matter of the epicenter die by 24 h after spinal cord injury (SCI), but their densities return to normal levels by 6 weeks. This repopulation is largely due to the proliferation of local progenitors that divide in response of CNS injury. Previous studies indicate that the secondary events that cause cell death after SCI also increase the local levels of several growth factors that stimulate the proliferation of these endogenous progenitors. We compared the spatial pattern of the post-injury up-regulation of the pro-mitotic growth factors with that of 5-bromodeoxyuridine (BrdU) incorporation to determine if each could play a role in proliferation. Three days after a standard contusive SCI or laminectomy, animals received intraperitoneal BrdU injections to label dividing cells and were perfused 2 h after the last injection. Immunohistochemistry for BrdU and basic fibroblast growth factor (FGF2) and in situ hybridization for ciliary neurotrophic factor (CNTF) and glial growth factor (GGF2) mRNA were used to compare the number of dividing cells with growth factor levels in sections 2 and 4 mm from the epicenter. All three growth factors are significantly up-regulated 3 days after SCI, when cell proliferation is maximal. The increase in GGF2 and FGF2 levels is highest in sections 2 mm rostral to the epicenter, mimicking BrdU incorporation. Addition of rhGGF2 to cultured cells isolated from the spinal cord 3 days after SCI increased the number of NG2+ glial progenitors. These data suggest that FGF2 and GGF2 may contribute to the spontaneous recovery observed after SCI by stimulating the proliferation of local progenitors that help repopulate the injured cord.

Theme: Development and regeneration
Topic: Neurotrophic factors: expression and regulation

Keywords: GGF2; FGF2; CNTF; Oligodendrocyte; Astrocyte; Glia; Precursor; Progenitor; BrdU; NG2

1. Introduction

Spinal cord injury can cause tragic disability due to focal cell death and loss of axonal transmission. While the impact of the initial injury destroys many of the local neurons and glia, cell loss is not limited to the primary mechanical insult, but it is exacerbated by secondary mechanisms that are activated in response to central nervous system (CNS) trauma [40]. The secondary injury affects all cells in the hypoxic penumbra of the lesion epicenter including neurons and glia. In addition to neuronal loss, approximately 50% of the astrocytes and oligodendrocytes in the spared white matter located at the site of injury die by 24 h after injury [18]. Despite the original cell loss, the densities of mature oligodendrocytes and astrocytes return to near normal levels by 6 weeks after injury [35,45]. Recovery of cell densities may be due in part to the proliferation of surviving glial cells [26]. The adult rat spinal cord contains glial progenitors [20] that could serve as a source of new and potentially myelinating oligodendrocytes. Following ischemic stroke injury, the density of glial progenitors begins to increase by 2 days, and this increase is accompanied by a restoration of oligodendrocyte and myelin density as early

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doi:10.1016/j.brainres.2005.05.071

as 2 weeks [39]. In a previous study of cell proliferation in the first week after SCI [46], we found that, following contusive SCI, 5-bromodeoxyuridine (BrdU) incorporation in surviving cells of the residual white matter is significantly increased as compared to laminectomy controls by 1 day after SCI and peaks at 3 days. Proliferation increased with proximity to injury epicenter, suggesting that factors released at the epicenter have a positive effect on cell proliferation. Three days after SCI, about one half of the dividing cells were NG2+ progenitors, and the other half was comprised of mature oligodendrocytes, astrocytes, and non-activated microglia. Six weeks later, cells labeled with BrdU 2–4 days after SCI comprised approximately one fourth of the mature oligodendrocytes and astrocytes [46].

These data suggest that secondary events after SCI not only cause cell death but also help restore chronic function by increasing the local levels of growth factors [21,27,42] that stimulate the proliferation of endogenous progenitors [9,23,31,]. Based on previous studies, we selected three growth factors that could stimulate the proliferation of glial progenitors following spinal cord injury in vivo and thus help in recovery of function. The published data on these growth factors show that they can stimulate proliferation of glial progenitors and that their levels are increased significantly in the week after injury to the CNS, when glial proliferation is maximal [26,46]. The factors are basic fibroblast growth factor (FGF2 [11]), ciliary neurotrophic factor (CNTF [21]), and glial growth factor (GGF2 [42]).

FGF2 stimulates the proliferation of glial progenitors [37] and mature oligodendrocytes [13] in vitro. Some evidence suggests that FGF2 also causes de-differentiation of mature oligodendrocytes to an immature state [16], while other studies point to proliferation of the GalC+ mature oligodendrocytes [13]. In our model of contusive SCI FGF2, mRNA [12] and protein [27] are increased within the week after injury, when most of the proliferation occurs [46].

CNTF also has a proliferative effect on glial populations in vitro. Addition of CNTF to the media of mature O1+ oligodendrocytes resulted in greater total cell numbers. Though initially the cultures contained only mature oligodendrocytes, after incubation with CNTF, both astrocytes and myelinating oligodendrocytes [34] were detected later, suggesting an initial reversion to a bipotential proliferative stage triggered by CNTF treatment.

GGF2, a member of the neuregulin family, triggers the proliferation of glial progenitors [9] and induces a phenotypic reversion in cultured oligodendrocytes, causing their return to a mitotic state [10]. In cultured glial progenitors, GGF promotes survival and stimulates proliferation while maintaining the cells in an immature phenotype [9]. Cultured adult optic nerve glial progenitors proliferate in response to GGF, but only in the presence of platelet derive growth factor (PDGF) and elevated cAMP levels [36]. These conditions are consistent with the molecular micro-environment of the ischemic injured spinal cord, where broken axons [5] and hypertrophic astrocytes [8] could contribute to extracellular GGF2 levels. Reactive astrocyte-derived PDGF [33] and trauma-induced cAMP elevation [43] could potentiate the effects of GGF2 to stimulate the proliferation of glial progenitors. In a chronic relapsing model for multiple sclerosis (MS), exogenously administered GGF/NRG protein delays relapse, reducing autoimmune demyelination and promoting remyelination [7]. This effect may be mediated by an increase in proliferation, as GGF2 is a strong mitogen for glial progenitors that help sheathe demyelinated axons [15].

We studied the spatiotemporal pattern of post-injury upregulation of each growth factor vs. that of BrdU incorporation to determine if there is a correlation. We also performed a dose–response study of the effects of recombinant human GGF2 (rhGGF2) on glial cells isolated from the injured spinal cord 3 days after SCI to determine if these cells are appropriately responsive to the factor.

2. Methods

2.1. SCI and post-surgical care

Animal protocols used for this study were reviewed and approved by the Animal Care and Use Committee at Georgetown University. Rats were housed in an animal care facility meeting all current USDA and NIH requirements under the supervision of a board certified veterinarian. Contusive spinal cord injury was performed as previously described [44] on female Sprague–Dawley rats weighing 225–250 g. Rats were anesthetized with chloral hydrate (360 mg/kg i.p.), and a laminectomy performed at the level of T8 to expose a circle of dura. Contusion was produced by dropping a 10 g weight from a height of 2.5 cm onto an impounder positioned on the dura. Controls for these experiments were age-, sex-, and weight-matched laminectomy rats that underwent the same surgical procedures without having the weight dropped. Assignment of rats in the experiment was done randomly in advance of surgery. The results reported are based on a total of 10 laminectomy control rats and 10 with SCI for the in vivo studies with 8 additional SCI rats used to prepare primary cell cultures. After injury, SCI animals received manual expression of their bladders until a reflex bladder was established. To prevent bladder-related health complications and to ease bladder expression, only female rats were used. Rats were housed in pairs to reduce stress from isolation and kept at 22–25 °C under conditions described above. Food and water were available ad libitum.

2.2. Behavioral analysis

To ensure that all experimental animals received similar degrees of injury and to monitor the rate of recovery, rats were tested for functional deficits according to the Basso Beattie and Bresnehan locomotion test (BBB [6]) and the Combined Behavior Score (CBS [14]) 24 h and 3 days after