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Research report

bFGF and EGF modulate trauma-induced proliferation and neurogenesis in juvenile organotypic hippocampal slice cultures

Alexandra Laskowski[a], Werner Schmidt[a], Klaus Dinkel[a,b], Mónica Martínez-Sánchez[b], Klaus G. Reymann[a,b,*]

[a]*Forschungsinstitut für Angewandte Neurowissenschaften (FAN), Leipziger Str. 44, 39120 Magdeburg, Germany*
[b]*Leibniz Institut für Neurobiologie, Projektgruppe Neuropharmakologie, Brenneckestr. 6, 39118, Magdeburg, Germany*

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Abstract

Since postnatal and adult mammalian brains have been shown to retain an ability to generate neurons from endogenous stem cells throughout life, these cells could play a central role in regeneration after neuronal loss. Therefore, we studied cell proliferation, glio- and neurogenesis respectively after brain injury in organotypic hippocampal slice cultures using a focal trauma by transecting Schaffer collaterals in the cornu ammonis (CA) 2 region mechanically. After determination of cell death using propidium iodide, neuroregenerative processes were quantitatively analyzed by various immunohistochemical techniques at different time points post injury. As this endogenous insult-induced neurogenesis is rather inefficient, we investigated if it can be enhanced by application of exogenous growth factors.

Exogenous basic fibroblast growth factor (bFGF) enhanced neurogenesis significantly in the dentate gyrus (DG) region. A neutralizing antibody against endogenous bFGF revealed a significant decrease of basal and trauma-induced proliferation. Reverse transcription polymerase chain reaction (RT-PCR) studies exhibited a downregulation of FGF messenger ribonucleic acid (mRNA) transcription after the antibody treatment. In contrast, epidermal growth factor (EGF) increased proliferation, but not neurogenesis. A combination of bFGF and EGF displayed an EGF-like effect on proliferation and no effect on neurogenesis. These results demonstrate, that in our model bFGF but not EGF sustains neurogenesis, whereas together the two growth factors permit an increased proliferation but not neurogenesis in organic hippocampal slice cultures.

Theme: Development and regeneration
Topic: Neurotrophic factors: biological effects

Keywords: Stem cells; Growth factors; Endogenous repair; In vitro; Regeneration; Degeneration

1. Introduction

After head trauma, neurons degenerate because of the direct effect of mechanical insult, or die as a result of the ischemia-induced changes. The neuronal death can be evoked by excitotoxicity caused through a rise of intracellular calcium, free radical formation, associated acidosis and other deleterious cellular processes involving the second messenger system [21]. Axotomized processes either show a Wallerian degeneration and disappear, or start to sprout [39], and reorganize. Alternatively, brain injury can also induce cell proliferation [31]. With recent observations of stem cell repositories in the brain [7], it was shown that different brain injuries induce proliferation [5,37], generation of new astrocytes [14], as well as new neurons [2,26,35].

The newly formed cells derive from proliferating neural progenitor cells, which are characterized as long-term

* Corresponding author. Leibniz Institut für Neurobiologie, Projektgruppe Neuropharmakologie, Brenneckestr. 6, 39118, Magdeburg, Germany. Fax: +49 391 6263 438.
E-mail address: reymann@ifn-magdeburg.de (K.G. Reymann).

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self-renewing and multipotent. Initially, it was assumed that neurogenic regions are limited to the dentate area (DG) of the hippocampus [28], and the lateral ventricle of the adult subventricular zone (SVZ) [6]. More recent studies report neurogenesis also in other brain regions like Ammon's horn [32], striatum, septum, thalamus, hypothalamus [29] or cortex [10,44]. The endogenous progenitor cells seem to be an auspicious tool for brain repair after injury, for review [16]. Arvidsson and colleagues found a 31-fold increase of dividing cells in the striatum of the ipsilateral side after stroke [2]. However, the efficiency of this cell replacement is limited, because the majority of newborn cells die shortly after generation [2].

The proliferative and neurogenic response to injury can be enhanced by administering exogenous growth factors (GF) (for review see [16]). Thus, basic fibroblast growth factor (bFGF) is known as a cell proliferation and neurogenesis inducing GF [12,18] in particular after brain injury [43].

In the present study, we used transection of Schaffer collaterals in organotypic hippocampal slice cultures (OHC) as an in vitro model for focal trauma. OHCs offer advantages over dissociated cultures in that they retain a three-dimensional structure and therefore preserve most of the synaptic and anatomical organization of the neuronal circuitry; and have functional characteristics similar to those found in vivo [9,38].

Here, we characterized proliferation and early neurogenesis after transecting the Schaffer collaterals with specific cell markers. Furthermore, we examined the influence of exogenous GF, like bFGF, epidermal growth factor (EGF) and their combination on cell proliferation and neuronal differentiation under basal conditions and after injury.

2. Material and methods

2.1. Hippocampal organotypic slice cultures (OHC)

Hippocampal cultures were prepared by the interface culture method, originally developed by Stoppini [38]. Briefly, 7 to 9-day-old Wistar (Harlan Winkelmann GmbH, Borchen, Germany) rats were decapitated, and their brains were quickly removed under sterile conditions. After isolation of the hippocampi, their dorsal halves were sectioned transversely at 350 μm by a McIlwain tissue chopper (The Mickle Laboratory Engineering Co., Guildford, UK). The resulting slices were inspected and separated in chilled, 100% O_2 saturated MEM-HANKS salt solution pH 7.35 (Biochrom, Berlin, Germany) containing 25 mM HEPES and 2 mM L-glutamine. Four to five slices were then placed on 25 mm diameter Anopore membrane inserts with 0.02 μm pore size (NUNC, Wiesbaden, Germany). The inserts were transferred to 6 well-culture plates (NUNC).

Each well contained 1.2 mL of tissue culture medium consisting of 50% MEM (Biochrom), 25% HBSS (Gibco, Eggenstein, Germany), 25% horse serum (Gibco), 350 mg/mL $NaHCO_3$ (Sigma, Deiβenhofen, Germany), 12.5 mM HEPES (Sigma), 0.5 mg/mL gentamycin (Biochrom) at pH 7.35. Slice cultures were maintained in a humidified incubator with 1% CO_2 at 37 °C. Culture medium was changed three times a week. Animals were handled in accordance to a protocol approved by our institutional animal care committee. All possible efforts were made to minimize animal suffering and the number of animals used.

2.2. Traumatic injury

After 7–9 days in vitro (DIV), the slices were selected based on their viability and morphology evaluated by propidium iodide (PI, Sigma) uptake and transmission microscopy respectively. Therefore, overnight incubation of PI staining in a final concentration of 0.5 μM was applied for preselection. PI enters cells with damaged membranes rapidly and binds to the DNA in the nucleus acting as a fluorescent marker for cell death. Only PI-negative and morphologically intact slices were selected for the experiments. After selection, the cultures were replaced to PI-free culture medium.

The trauma was set afterwards by transection of the Schaffer collaterals with a scalpel in the CA2 region (cut from the tip of the upper blade of the dentate gyrus through the CA2 area). Therefore, the inserts were put in a petri dish under the sterile conditions of a laminar box. The injury was set under a 20× magnification.

2.3. Degeneration

Cell death was evaluated by cellular uptake of PI after 2, 4 and 6 days respectively. Cultures were incubated with PI-containing medium with a final concentration of 10 μM for 2 h at 37 °C (Fig. 1A). The fluorescent dye intercalated into the DNA of cells that lost membrane integrity and therefore represents a marker of cell death. Afterwards, the slice was excited with a 510–560 nm light and the emitted fluorescence acquired at 610 nm using a rhodamine filter on an inverted fluorescence microscope (Eclipse TE 300, Nikon). Images were taken using a CCD camera and analyzed on a PC with an image analysis software (LUCIA, Nikon). The uptake of PI to identify degenerating neurons in OHCs has been established by Pozzo Miller [30]. Damage was given as % of the total CA area labeled PI-positive.

2.4. Cell proliferation

Proliferating cells and their progeny were detected by incorporation of the thymidine analogue BrdU into the DNA of cells during S-phase of the cell cycle [27].