Brain Research 1650 (2016) 60–72

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/brainres

Research report

Association between apoptotic neural tissue and cell proliferation in the adult teleost brain

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ARTICLE INFO

Article history:
Received 12 May 2016
Received in revised form
18 August 2016
Accepted 23 August 2016
Available online 24 August 2016

Keywords:
Neuro-regeneration
Tilapia
BrdU
TUNEL
Habenula

ABSTRACT

Injury to neuronal tissues in the central nervous system (CNS) of mammals results in neural degeneration and sometime leads to loss of function, whereas fish retain a remarkable potential for neuro-regeneration throughout life. Thus, understanding the mechanism of neuro-regeneration in fish CNS would be useful to improve the poor neuro-regenerative capability in mammals. In the present study, we characterized a neuro-regenerative process in the brain of a cichlid, tilapia, *Oreochromis niloticus*. Morphological observations showed that the damaged brain region (habenula) successfully regrew and re-innervated axonal projections by 60 days post-damage. A fluorescent carbocyanine tracer, DiI tracing revealed a recovery of the major neuronal projection from the regenerated habenula to the interpenduncular nucleus by 60 days post-damage. TUNEL assay showed a significant increase of apoptotic cells ($\sim$234%, $P < 0.01$) at one day post-damage, while the number of bromodeoxyuridine (BrdU)-positive proliferative cells were significantly increased ($\sim$92%, $P < 0.05$) at 7 days post-damage compared with sham-control fish. To demonstrate a potential role of apoptotic activity in the neuro-regeneration, effects of degenerative neural tissue on cell proliferation were examined *in vivo*. Implantation of detached neural but not non-neural tissues into the cranial cavity significantly ($P < 0.01$) increased the number of BrdU-positive cells nearby the implantation regions at 3 days after the implantation. Furthermore, local injection of the protein extract and cerebrospinal fluid collected from injured fish brain significantly induced cell proliferation in the brain. These results suggest that factor(s) derived from apoptotic neural cells may play a critical role in the neuro-regeneration in teleost brain.

1. Introduction

In the central nervous system (CNS) of mammals, adult neurogenesis mainly occurs in the subventricular zone of the lateral ventricles and in the subgranular zone (SGZ) of the dentate gyrus in the hippocampus, with low proliferative activity evident in the hypothalamus (Jin et al., 2003; Kokoeva et al., 2005; Leung et al., 2007; Yuan and Arias-Carrion, 2011). However, the cells generated are limited in terms of their survival, differentiation and/or integration into the existing neural circuitry (Chapouton et al., 2007; Jin et al., 2006). It is estimated that as low as 0.2% of newly differentiated neurons contribute to the reconstruction of damaged neural circuitry (Arvidsson et al., 2002; Magavi et al., 2000). The presence of inhibitory factors and absence of a permissive environment further hinder neuroregeneration in the adult mammalian CNS (Sirbulescu et al., 2009). Nevertheless, several studies have demonstrated successful integration of newly differentiated neurons into the existing neural circuitry of the mammalian CNS (Arvidsson et al., 2002; Jin et al., 2004, 2006). This suggests that the mammalian CNS retains neuroregenerative capability.

In the brains of patients who have suffered an ischemic stroke, new neuronal progenitor cells are observed only in the SGZ but not in the stroke-damaged area (Arvidsson et al., 2002). Developing and differentiating neurons migrate from the SGZ to the stroke-damaged area (Arvidsson et al., 2002). Similar observations have been reported in the brains of patients with neurodegenerative disorders, such as Alzheimer's disease and Huntington's disease (Jin et al., 2004; Winner et al., 2011). This suggests that damaged neural tissues may release signals that stimulate the generation of new neurons and induce them to migrate and repair damaged areas of the brain. Neural loss also leads to the loss of connectivity with neighboring cells and existing neural circuits, which is also considered to stimulate neurogenic sites to generate new cells (Hsieh and Gage, 2004; Winner et al., 2011).

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http://dx.doi.org/10.1016/j.brainres.2016.08.033
0006-8993/

Degenerating neurons have been shown to induce the generation of new cells through the release of certain growth factor(s) (Gould and Tanapat, 1997). However, signaling mechanisms underlying this stimulation of the generation and migration of new cells to damaged sites in the mammalian CNS remain unclear.

In contrast, most non-mammalian vertebrates exhibit extensive cell proliferation in the CNS (Chapouton et al., 2007; Minelli et al., 1987). In teleost fish, several studies have demonstrated the complete reconstitution of the brain structure, even after its partial removal (Bernstein, 1967; Lindsey and Tropepe, 2006; Marón, 1963; Segaar, 1965). Conversely, although the amphibian CNS exhibits a similar neuroregenerative capability, neuroregeneration occurs only from larval life until immediately after metamorphosis and is absent in the adult CNS (Filoni and Margotta, 1971; Srebro, 1965). In fish, zones of cell proliferation are widely distributed throughout the CNS (Kaslin et al., 2008) and retain their neuroregenerative capability after injury or damage even in adults (Zupanc, 2006). Generally, neuroregeneration in the fish CNS starts with the generation of a large number of mitotic cells in zones of cell proliferation located on the ventricular surfaces of the brain (Takeda et al., 2008). These newly generated cells migrate towards injured sites to participate in the regeneration process, subsequently differentiating into new neurons, glia and supporting cells (Kaslin et al., 2008).

Effective regenerative and reparative roles after brain injury displayed in fish raises the possibility of developing therapeutic strategies to harness endogenous neurogenic capacity in mammalian CNS. Damaged or dead cells removal is among the earliest response after brain injury (Aertker et al., 2016). Necrosis and apoptosis are the main mechanisms involved for dead cell clearance (Elmore, 2007). Necrosis is likely to cause inflammatory response at the site of injury due to the release of cellular components which subsequently attracts high number of macrophages to initiate phagocytosis to clear off cellular debris in mammals (Elmore, 2007). Macrophages stimulate subsequent activation and accumulation of astrocyte at the injury site which prevent the ingrowth of nerve fibers and migration of cells into the injured site (Burda et al., 2016; Elmore, 2007; Hausmann, 2003). On the other hand, the cell death removal mechanism for fish is predominantly apoptosis (Zupanc et al., 1998). The apoptosis mechanism causes less harm to surrounding cells as minimal immune response is being activated (Charriaut-Marlangue et al., 1996). Hence, the negative side effects from astrocyte are less in apoptosis and believed to be more efficient in neuroregeneration (Zupanc and Zupanc, 2006). Additionally, high proliferative activity is counterbalanced by apoptosis during brain injury in fish (Zupanc, 1999). This suggests that cell apoptosis process might have an essential role in inducing proliferative activity for neuroregeneration. However, the mechanism underlying how cell apoptosis induces cell proliferation is still unknown.

In this study, we examined the process of mechanical damage-induced neuroregeneration in the brain of the cichlid tilapia (*Oreochromis niloticus*). Stereotaxic coordinates for the tilapia brain has previously been developed and used for lesioning of different brain regions (Parhar, 1990; Yamamoto et. al., 1998). Adult tilapia has relatively bigger brain size as compared to zebrafish, commonly used model, which increases efficiency and reproducibility of the specific brain region removal. Also, long survival with insertion of a guided cannula into the tilapia brain has demonstrated strong resistance to brain lesioning in our previous studies (Ogawa et al., 2006; Yamamoto et al., 1997). For our mechanical damage experiments, we chose the habenula, a paired structure located in the diencephalon, for the following reasons: it is highly evolutionarily conserved among vertebrates (Bianco and Wilson, 2008); it is a region of cell proliferation (Kaslin et al., 2008) and has a relatively compact structure located on the surface of the brain, allowing us to operate with minimal damage to other brain regions. Using this model, we first characterized the neuroregenerative process in the brain of tilapia using bromodeoxyuridine (BrdU) and terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assays. Using the fluorescent carbocyanine tracer DiI, we examined the regeneration of neural innervation from the habenula to the interpeduncular nucleus (IPN). To examine the potential role of apoptosis in stimulating proliferative cells for neuroregeneration, we analyzed the effect of the implantation of detached neural tissues on cell proliferation in the brain. Finally, to confirm that apoptotic tissues are a source of stimulants that induce cell proliferation, we administered proteins extracted from apoptotic neural tissues and cerebrospinal fluid (CSF) extracted from the injured brain into the intact brain and examined its effect on cell proliferation.

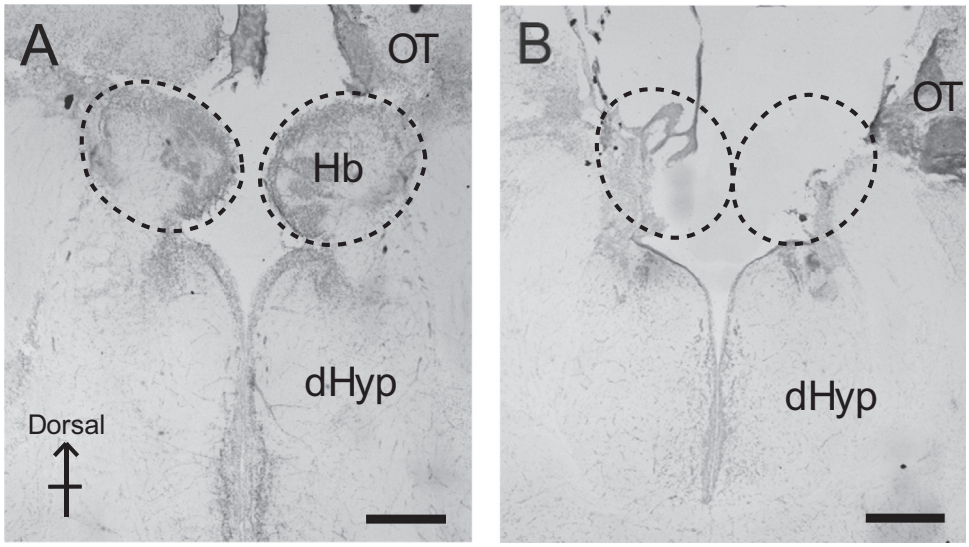

Fig. 1. Photomicrographs of cresyl violet staining of coronal sections of the tilapia brain before (A) and after (B) habenula damage. Dotted circles indicate the location of the habenula. Abbreviations: dHyp: dorsal hypothalamus, Hb: habenula and OT: optic tectum Scale bars: 200 μm.