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BRAIN RESEARCH

Research Report

Endogenous neurogenesis in the hippocampus of developing rat after intrauterine infection

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ABSTRACT

Perinatal infection is a major cause of neonatal neurologic morbidity. The goal of this study is to examine the effects of intrauterine infection on hippocampal neurogenesis and self-repair during early developmental stages. The animals were randomly divided into two groups: *E. coli* infected group and control group. Neurogenesis was examined by incorporation of BrdU, a marker of proliferating cells and their progeny. Rats were sacrificed on P3, P7, P14 and P28, and their brains were prepared for histological analysis of cell proliferation. To evaluate hippocampus neurogenesis, rats were sacrificed on P7 and P28, and their brains were prepared for evaluation of newly generated neural stem cells using double labeling of BrdU and Nestin, newly formed neurons using double labeling of BrdU and NeuN, and newly formed astrocytes using double labeling of BrdU and GFAP. In intrauterine *E. coli* infected group, there was significant increase in numbers of BrdU-labeled cells (about 2-fold at P7) than that of the control group ($P<0.05$). Confocal microscopy showed that there was a significant difference in BrdU/Nestin coexpression between the control and *E. coli* infected groups ($P<0.01$). Evaluation of the phenotype of the surviving cells showed that *E. coli* infected and control groups had a similar proportion of neuronal and glial differentiation. No significant difference was found in the percentage of newborn cells expressing neuronal and glial phenotype in the control and *E. coli* infected groups ($P>0.05$). Real-time RT-PCR and Western blot analysis showed that there was a significant increase of BDNF, TrkB, p-Akt and Survivin mRNA and protein expression during postnatal 7 days in the *E. coli* infected group ($P<0.05$). Our results suggest that endogenous neurogenesis may occur in hippocampus in early postnatal period and may be enhanced by neonatal inflammation reactive syndrome. The PI3K/Akt signaling pathway may be involved in regulation of BDNF and is important in the potential activation of neuroprotective and repair pathways during critical time windows of hippocampal development.

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1. Introduction

Perinatal infection occurs during a critical period of brain growth and development. White matter and its resident oligodendrocyte progenitors are especially vulnerable to perinatal brain injuries (Back et al., 2002). Intrauterine infection can cause white matter damage (WMD), the most common cause of cerebral palsy (CP) (Ness et al., 2001; Yuan et al., 2005). WMD in the developing brain is therefore a major cause of cognitive impairment that often persists into adulthood. Current therapeutic strategies are aimed mainly at preventing brain damage, however, there are no effective methods to reverse the damage (Joannides et al., 2007). Moreover, accumulating evidence suggests that most of the insults occur in the utero, hence preventive methods may be difficult and limited in application, demanding regenerative strategies to be pursued to reduce the associated morbidity. Neurogenesis arising from populations of neural progenitors that persist in neurogenic niches of the sub-ventricular zone (SVZ) and sub-granular zone (SGZ) of the dentate gyrus (DG) is now a universally accepted feature of the postnatal and adult mammalian brain (Gould, 2007). Adult mammalian brain also has some capacity for endogenous regeneration after various kinds of insults that lead to loss of neurons, such as hypoxia–ischemia, acute seizures, trauma and viral infection (Arvidsson et al., 2002; Fatemi et al., 1999, 2002; Goings et al., 2004; Magavi et al., 2000; Parent and Silverstein, 2007). Studies have also shown that endogenous neurogenesis after ischemic injuries produces new cells that integrate into neural networks and participate the recovery from neurological deficit (Nakatomi et al., 2002). These findings raised the possibility of repairing damaged circuits by recruiting latent regenerative potential.

In neonatal brains, hypoxia–ischemia has been shown to increase hippocampal precursor cell survival (Bartley et al., 2005), and endogenous neurogenesis can be modulated by various extrinsic and intrinsic factors (Bruel-Jungerman et al., 2006). However, there are no studies about the effect of intrauterine infection during the life stages when hippocampal neurogenesis is most prominent. Therefore harnessing capabilities of neurotrophic factors to amplify neurogenesis is a goal of neonatal WMD recovery therapy. Our previous studies demonstrated that oligodendrocyte loss and axonal degeneration occurred in periventricular white matter after intrauterine *E. coli* infection and reactive astrogliosis was a characteristic response of astrocytes to inflammation and damage (Yu et al., 2004). We reported that proinflammatory cytokines and chemokines might be a mechanism mediating the neonatal WMD after the intrauterine *E. coli* infection. Additionally, in the *E. coli*-infected animal model, the neuroprotective effect of erythropoietin (EPO) on WMD led to increased oligodendrocytes survival, increased preservation of axons, and decreased generation of reactive astrogliosis, as accessed by CNPase, NF and GFAP protein expression (Shen et al., 2009). Given the mechanism of intrauterine *E. coli* infection, the aim of the current study was to explain what role endogenous neurogenesis plays in neonatal WMD plasticity after intrauterine infection. In this study, newborn cells were labeled by bromodeoxyuridine (BrdU) incorporation. The cells that survived the process of maturation and differentiation were quantified by double-labeling immunofluorescence. Using cell lineage markers for neurons and astrocytes, and by quantifying the co-localization of such markers with the BrdU labeled nuclei, we addressed inherent cell-lineage commitment patterns in hippocampal derived cells in intrauterine infection comparatively with the control.

Brain-derived neurotrophic factor (BDNF), a cognate ligand for the tyrosine kinase receptor B (TrkB) receptor, mediates neuronal survival, differentiation, synaptic plasticity and neurogenesis. PI3K/Akt signaling pathway is a classic anti-apoptotic pathway that urges survival signal transduction. In adult rats, BDNF, TrkB and Akt signals have been detected in neuronal cells following central nervous system (CNS) trauma or ischemia (Ferrer et al., 2001; Luikart et al., 2008; Schäbitz et al., 2000). However, no studies to date have examined whether the PI3K/Akt signaling pathway is involved in hippocampal neurogenesis after intrauterine *E. coli* infection. Therefore, using *E. coli*-infected animal model, we investigated whether BDNF, TrkB, p-Akt and Survivin expression is significantly increased in hippocampus for regenerative potential and their neuroprotective role after intrauterine *E. coli* infection.

2. Results

2.1. *Effect of intrauterine infection on cell proliferation*

Newborn cells in the developing rat dentate gyrus can be quantified by immunostaining of BrdU incorporated into the nuclei of dividing cells (Gage, 2002; Gould and Gross, 2002). In our study, animals were sacrificed on P3, P7, P14 or P28 after 3 consecutive injection of BrdU for cell proliferation analysis. The typical morphology of BrdU-labeled nuclei is dark and round in shape, and frequently with granular nuclei (Figs. 1A–B). The effect of intrauterine infection on dentate gyrus neurogenesis was evaluated by comparing the average number of BrdU-positive cells per area in control (n=5) and in *E. coli* infected rats (n=5). Overall repeated ANOVA analyses showed that there were significant differences from P3 to P28 between control and *E. coli* infected groups ($P<0.05$ on P3, 7, 14). In intrauterine *E. coli* infected group, there was a significant increase in numbers of BrdU-labeled cells than that of the control group (P3: 870.00±25.50, 620.00±25.50, $P<0.05$; P7: 2110.00±55.23, 1150.00±55.23, $P<0.05$; P14: 1310.00±23.89, 920.00±23.89, $P<0.05$). And on P28, there were no significant differences between *E. coli* infected and control groups (*E. coli* infected, 844.00±19.37; control, 792.00±19.37, $P>0.05$) (Fig. 1C). These results indicate that intrauterine *E. coli* infection may promote cell proliferation.

2.2. *Effect of intrauterine infection on neuronal survival and differentiation*

In these two groups, newborn cells were also analyzed for coexpression of BrdU and Nestin (Fig. 2), a marker of intermediate neurofilament. There was a significant difference in BrdU/Nestin coexpression between the control (30.35±1.28%) and *E. coli* infected (44.09±0.38%) groups ($t=10.39$; $P<0.01$) (Fig. 2D). Results showed that intrauterine *E. coli* infection was