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A role for interleukin-1β in determining the lineage fate of embryonic rat hippocampal neural precursor cells

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ABSTRACT

Neurogenesis occurs in the hippocampus of the developing and adult brain due to the presence of multipotent stem cells and restricted precursor cells at different stages of differentiation. It has been proposed that they may be of potential benefit for use in cell transplantation approaches for neurodegenerative disorders and trauma. Prolonged release of interleukin-1β (IL-1β) from activated microglia has a deleterious effect on hippocampal neurons and is implicated in the impaired neurogenesis and cognitive dysfunction associated with aging, Alzheimer's disease and depression. This study assessed the effect of IL-1β on the proliferation and differentiation of embryonic rat hippocampal NPCs *in vitro*. We show that IL-1R1 is expressed on proliferating NPCs and that IL-1β treatment decreases cell proliferation and neurosphere growth. When NPCs were differentiated in the presence of IL-1β, a significant reduction in the percentages of newly-born neurons and post-mitotic neurons and a significant increase in the percentage of astrocytes was observed in these cultures. These effects were attenuated by IL-1 receptor antagonist. These data reveal that IL-1β exerts an anti-proliferative, anti-neurogenic and pro-gliogenic effect on embryonic hippocampal NPCs, which is mediated by IL-1R1. The present results emphasise the consequences of an inflammatory environment during NPC development, and indicate that strategies to inhibit IL-1β signalling may be necessary to facilitate effective cell transplantation approaches or in conditions where endogenous hippocampal neurogenesis is impaired.

Introduction

It is well established that the prototypic pro-inflammatory cytokine interleukin-1β (IL-1β) is a key mediator of cell death in acute neurodegenerative conditions, such as stroke and head injury (Allan et al., 2005). Likewise, sustained microglial activation in response to chronic stress, infection, toxins, age or infiltrating cytokines from the periphery is responsible for prolonged release of IL-1β from microglia, which can affect neuronal survival, growth, synaptic transmission and hippocampal-dependent memory processes (Yirmiya and Goshen, 2011). In microglia, IL-1β is synthesised as inactive pro-IL-1β and is cleaved by caspase-1 to generate mature active IL-1β. The responses to IL-1β are initiated by its binding to the cell surface IL-1 type-1 receptor (IL-1R1) (Greenfeder et al., 1995). The resulting signalling cascade releases nuclear factor kappa-B (NFκB) from the inhibitory kappa B (IκB) protein, allowing it to translocate to the nucleus, and alter gene transcription (DiDonato et al., 1997). IL-1β can also bind to a decoy receptor, interleukin-1 type-2 receptor (IL-1R2) which does not induce signalling due to the lack of an intracellular domain (McMahan et al., 1991). The naturally occurring receptor antagonist of IL-1β, IL-1 receptor antagonist (IL-1RA) is structurally similar to IL-1β and can bind to IL-1R1 with almost equal affinity to that of IL-1β, however it does not induce any intracellular response (Hannum et al., 1990). Instead, IL-1RA prevents the interaction of IL-1β with IL-1R1. In the rodent brain, the density of IL-1R1 is highest in the hippocampus (Farrar et al., 1987; Parnet et al., 1994). It is established that IL-1R1 is present on mature hippocampal rat neurons (Nolan et al., 2004) and that IL-1β signalling causes the death of these neurons (Maher et al., 2005). While there is also evidence to demonstrate that at low concentrations IL-1β contributes to the normal physiological functions of LTP and spatial memory (Schneider et al., 1998), there is an accumulating array of data showing that higher concentrations of IL-1β inhibits learning, memory and long-term potentiation (Depino et al., 2004; Nolan et al., 2005; Yirmiya et al., 2002). Hippocampal IL-1β-mediated cognitive impairment is also associated with aging (Barrientos et al., 2009; Nolan et al., 2005), Alzheimer's disease (AD) (Licastro et al., 2000), stress (Goshen and Yirmiya, 2009; Vereker et al., 2001) and depression (Goshen et al., 2007).

Abbreviations: AD, Alzheimer's disease; bFGF, basic fibroblast growth factor; BrdU, 5′-bromo-deoxyuridine; DG, dentate gyrus; DIV, days *in vitro*; DCX, doublecortin; DMEM, Dulbecco's modified eagle's medium; E, embryonic age; EGF, epidermal growth factor; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GFAP, glial fibrillary acidic protein; IκB, inhibitory kappa B; IL-1α, interleukin-1α; IL-1β, interleukin-1β; IL-1R1, interleukin-1 type-1 receptor; IL-1R2, interleukin-1 type-2 receptor; IL-1RA, IL-1 receptor antagonist; IL-1RAcP, interleukin-1 receptor accessory protein; IL-6, interleukin-6; LPS, lipopolysaccharide; NFκB, nuclear factor kappa B; NPC, neural precursor cell; PBS-T, phosphate buffered saline-Tween 20; TNFα, tumour necrosis factor-α.

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The embryonic brain and defined regions of the adult mammalian brain, including the hippocampus, are capable of generating new neurons from multipotent stem cells (Gage, 2000). These cells can self-renew through symmetric division, or give rise to lineage-restricted daughter cells through asymmetric division. Lineage-restricted cells still maintain progenitor properties, they are capable of self-renewal, through symmetric division or can differentiate to become mature and functioning cells of one distinct lineage (neuronal, astroglial or oligodendroglial). Together, central nervous system stem cells and all progenitor types are broadly defined as neural precursor cells (NPCs). Numerous studies have proposed that NPCs may be therapeutically beneficial for a wide range of central nervous system disorders, including neurodegenerative disorders, demyelinating disorders, stroke, and trauma (Dyson and Barker, 2011; Kan et al., 2010; Lindvall and Kokaia, 2010; Martino et al., 2010). Moreover, the knowledge that neurogenesis also occurs in the adult hippocampus raises the possibility that endogenous repair by these cells may also be feasible. Indeed, adult hippocampal neurogenesis is involved in learning and memory (Gould et al., 1999; Shors et al., 2001), while impaired adult neurogenesis has been implicated in the cognitive decline observed in animal models of AD (Demars et al., 2010; Verret et al., 2007) and depression (Jacobs et al., 2000). Observations from transplantation experiments have elucidated that NPC biology is regulated by the characteristics of the microenvironment in which they reside (Herrera et al., 1999; Ma et al., 2005). Consequently, an array of extrinsic signals has now been identified that regulates hippocampal neurogenesis (Ma et al., 2010), and information has accumulated proposing pro-inflammatory cytokines as key players in modulating NPCs development and survival. For example, in the adult hippocampus, interleukin-6 (IL-6) has been reported to reduce the number of proliferating cells *in vivo* (Vallieres et al., 2002) and decrease the number of newly-born neurons from adult hippocampal cultures *in vitro* (Monje et al., 2003). Tumour necrosis factor-α (TNFα) is reported to have a negative effect on both embryonic and adult rat hippocampal neurogenesis, as shown by a reduction in neural precursor cell proliferation *in vitro and in vivo* (Iosif et al., 2006; Keohane et al., 2010). It has been shown that adult mice chronically exposed to IL-1 have impaired hippocampal neurogenesis (Goshen et al., 2007) and that IL-1β-induced inhibition of proliferation of adult rat hippocampal progenitors was reversed by IL-1RA (Koo and Duman, 2008). These studies support the premise of an anti-neurogenic role for IL-1β and introduce a potential for anti-inflammatory therapy for the restoration of impaired hippocampal neurogenesis, and hence disorders involving a deficit in adult hippocampal neurogenesis. While the effect of IL-1β on the proliferation of adult hippocampal NPCs has been well studied, little is known about the effect of IL-1β on embryonic hippocampal NPC lineage restriction, differentiation and thus cell development. Evidence now indicates that adult neurogenesis replicates essential mechanisms involved in NPC development in the embryonic brain (Hodge and Hevner, 2011; Kriegstein and Alvarez-Buylla, 2009; Liu and Zhao, 2009; Qu and Shi, 2009). Therefore, examining the effect of the pro-inflammatory cytokine IL-1β on embryonic hippocampal neurogenesis will contribute to the information needed to optimise the proliferation, migration, differentiation, and survival of NPCs for the development of cell replacement therapies, and also help us to understand the effects of inappropriate inflammation in the adult brain.

Results

Proliferating and differentiated hippocampal NPCs express IL-1R1

To assess whether embryonic rat hippocampal NPCs could be a target for IL-1β, we analysed the expression of IL-1R1 and IL-1R2 on these cells. RT-PCR analysis of NPC cultures treated with IL-1β (10 ng/mL) for 7 DIV while the NPCs are proliferating demonstrated that IL-1R1 mRNA was expressed on untreated and IL-1β-treated NPCs (Fig. 1A). Real-time PCR revealed that IL-1β treatment significantly increased IL-1R1 mRNA expression ($p<0.001$) (Fig. 1B). IL-1R2 mRNA was expressed at very low levels and IL-1β had no effect on IL-1R2 mRNA expression (Fig. 1A). The expression of IL-1R1 protein on cells from untreated and IL-1β treated cultures under proliferation conditions was demonstrated immunocytochemically (Fig. 1C). The percentage composition of cells expressing IL-1R1 was not altered by IL-1β treatment (Fig. 1D), however IL-1β induced an increase in the intensity of IL-1R1 expression within these cells (Fig. 1C). The expression of IL-1R1 protein on untreated nestin-positive and BrdU-positive NPCs under proliferation conditions was also demonstrated by immunocytochemical double-staining (Fig. 1C). These data indicate that proliferating NPCs could be susceptible to an inflammatory insult which may influence their capability to proliferate or differentiate. We next examined IL-1R1 mRNA expression in cells under differentiation conditions. RT-PCR for IL-1R1 and IL-1R2 was carried out on hippocampal NPCs that had been exposed to IL-1β (10 and 100 ng/mL) for 2 or 7 DIV under differentiating conditions (Fig. 1E). Real-time PCR analysis revealed that both concentrations of IL-1β significantly increased IL-1R1 mRNA expression after 2 ($p<0.01$) and 7 ($p<0.001$) DIV (Fig. 1F). IL-1R2 was not expressed in hippocampal NPCs under basal conditions, for 2 or 7 DIV under differentiating conditions, and IL-1β (10 and 100 ng/mL) did not induce its expression (Fig. 1E). Immunocytochemistry revealed that IL-1R1 protein was present on cells in untreated and IL-1β treated cultures after 7 DIV under differentiation conditions. Almost all cells expressed IL-1R1, and IL-1β treatment induced an increase in IL-1R1 protein expression in these cells (Fig. 1G). The expression of IL-1R1 protein on untreated DCX-positive cells (newly-born neurons), βIII-tubulin-positive cells (post-mitotic neurons) and GFAP-positive cells (astrocytes) after 7 DIV under differentiation conditions was demonstrated immunocytochemically (Fig. 1H). These results suggest that IL-1β has the potential to influence the lineage fate of NPCs as well as the function of their differentiated progeny.

IL-1β inhibits neurosphere formation, NPC proliferation and cell survival

To investigate the effect of IL-1β on embryonic rat hippocampal NPC neurosphere formation, cells were cultured in the presence or absence of IL-1β (10 ng/mL) under proliferating conditions, and neurosphere size was quantified over 7 days. Neurospheres formed on day 1, and both untreated and IL-1β-treated neurospheres increased in size during the 7 DIV (Fig. 2A and B). IL-1β treatment significantly decreased neurosphere circumference compared to untreated cultures in a time-dependent manner. Specifically, there was no significant difference in neurosphere circumference between the IL-1β and untreated cultures on days 1 to 3, however by day 4, IL-1β significantly decreased neurosphere circumference compared to untreated cultures ($p<0.01$) and this effect was maintained at all days analysed until 7 days ($p<0.001$) (Fig. 2A). We subsequently quantified the total cell number in T25 flasks after 4 DIV, as this was the earliest time point at which IL-1β was exerting an effect. The total cell number dissociated from floating neurospheres cultured in the presence or absence of IL-1β was analysed to determine if the observed decrease in neurosphere size was due to a decreased number of cells. IL-1β-treated neurosphere cultures had significantly fewer cells after 4 days under proliferating conditions ($p<0.01$) (Fig. 2C). We assessed if this decrease was due to reduced cell proliferation, reduced cell survival or as a result of the progenitor cells starting to differentiate. To measure cell proliferation, BrdU incorporation was quantified in neurospheres after 4 DIV. We found that IL-1β treatment for 24 hours after 4 DIV significantly