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Research Report

Caspase activation contributes to astrogliosis

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ABSTRACT

Caspases, a family of cysteine proteases, are widely activated in neurons and glia in the injured brain, a response thought to induce apoptosis. However, caspase activation in astrocytes following injury is not strongly associated with apoptosis. The present study investigates the potential role of caspase activation in astrocytes with another characteristic response to neural injury, astrogliosis. Caspase activity and morphological and biochemical indices of astrogliosis and apoptosis were assessed in (i) cultured neonatal rat astrocytes treated with astrogliosis-inducing stimuli (dibutryl cAMP, β-amyloid peptide), and (ii) cultures of adult rat hippocampal astrocytes generated from control and kainate-lesioned rats. The effects of broad spectrum and specific pharmacological caspase inhibitors were assessed on indicators of astrogliosis, including stellate morphology and expression of glutamine synthetase and fibroblast growth factor-2. Reactive neonatal and adult astrocytes demonstrated an increase in total caspase activity with a corresponding increase in the expression of active caspase-3 in the absence of cell death. Broad spectrum caspase inhibition with zVAD significantly attenuated increases in glutamine synthetase and fibroblast growth factor-2 in the reactive astrocytes. In the reactive neonatal astrocyte cultures, specific inhibition of caspases-3 and -11 also attenuated glutamine synthetase and fibroblast growth factor-2 expression, but did not reverse the morphological reactive phenotype. Astrogliosis is observed in all forms of brain injury and despite extensive study, its molecular triggers remain largely unknown. While previous studies have demonstrated active caspases in astrocytes following acute brain injury, here we present evidence functionally implicating the caspases in astrogliosis.

1. Introduction

Damage to the brain, be it mechanical trauma, disease, or even normal aging, elicits a robust astrocytic reaction termed astrogliosis (Pekny and Nilsson, 2005). Astrogliosis can be considered an attempt to restore homeostasis in the damaged brain through important functions including glial scar formation, regulation of immune responses, and the modulation of neuronal survival and neurite outgrowth (Sofroniew, 2009). Morphologically, astrogliosis is characterized by changes including hypertrophy, stellation and proliferation, in addition to numerous biochemical changes, which in turn alter neuronal function

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Abbreviations: Aβ, β-amyloid; dBcAMP, dibutryl cyclic adenosine monophosphate; FGF-2, fibroblast growth factor-2; GFAP, glial fibrillary acidic protein; GS, glutamine synthetase

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and viability (Pekny and Nilsson, 2005). Reactive astrocytes express proteins capable of both beneficial and detrimental effects on parameters of neuronal health, outcomes of which are dependent upon factors such as the age of the organism and the type and extent of injury (Sofroniew, 2009). Since astrogliosis can result in such functionally diverse outcomes on neuronal survival, a thorough understanding of the signal transduction pathways regulating astrocyte reactivity may provide insight into normal and pathological astrocytic responses to injury.

Caspase activation is a widespread event following disease or damage to the brain, not only present in neurons and microglia, but also observed in astrocytes (Acarin et al., 2007; Benjelloun et al., 2003; Mouser et al., 2006; Narkilahti et al., 2003; Su et al., 2000; Villapol et al., 2008). Caspases are cysteine proteases classified as either initiator caspases, including caspase-2, -8, -9 and -10, or executioner caspases, including caspase-3, -6 and -7. Initiator caspases proteolytically cleave and activate executioner caspases. In turn, executioner caspases proteolytically cleave and degrade structural proteins, signaling molecules, and DNA repair enzymes (Kumar, 2006). While the caspase family is predominantly associated with the processing of pro-inflammatory cytokines and the execution of apoptosis (Chang and Yang, 2000), various non-apoptotic functions including modulation of cell proliferation, differentiation and cell spreading have been described in a variety of cell types (McLaughlin, 2004; Schwerk and Schulze-Osthoff, 2003). In the brain, the caspase family have also been implicated in neuronal (Fernando et al., 2005) and glial differentiation (Oomman et al., 2006), neuronal cytoskeletal remodeling (Acarin et al., 2007; Rohn et al., 2004), synaptic plasticity (Dash et al., 2000), axon guidance and neurite outgrowth (Campbell and Holt, 2003; Chan and Mattson, 1999) as well as contributing to neuronal survival following preconditioning (Garnier et al., 2003; McLaughlin, 2004; Tanaka et al., 2004).

While some studies attribute caspase activation in astrocytes to glial degeneration (Su et al., 2000), several studies have also demonstrated that astrocytic expression of active caspases is not always associated with cell death following ischemic/excitotoxic damage (Acarin et al., 2007; Duran-Vilaregut et al., 2010; Narkilahti et al., 2003; Villapol et al., 2008; Wagner et al., 2011) and traumatic brain injury (Beer et al., 2000). Considering that gliosis, not cell death, is the principal astrocytic response to injury, it is possible that caspase activation in astrocytes may exert non-apoptotic functions that contribute to astrogliosis.

To investigate a possible non-apoptotic function of caspases in the regulation of astrogliosis, we examined caspase activity and indices of reactivity in rat astrocyte cultures. Experiments were conducted in two different culture paradigms. First, we utilized primary cultures of neonatal rat astrocytes that were exposed to β-amyloid (Aβ), an aggregating peptide that is associated with astrogliosis in both Alzheimer's disease brain (Rodríguez et al., 2009) and culture models. Treatment of astroglial cultures with Aβ typically does not result in cell death (Kato et al., 1997; Pike et al., 1994) but causes morphological features of gliosis (Abe et al., 1997; Canning et al., 1993; Hu et al., 1998; Pike et al., 1994; Salinero et al., 1997) as well as increased glial fibrillary acid protein (GFAP) immunoreactivity (Hu et al., 1998; Pike et al., 1994) and elevated levels of several cytokines, growth factors, and enzymes (Araujo and Cotman, 1992; Hu et al., 1998; Pike et al., 1994, 1996a). For comparison, we also treated cultures with dibutryl cyclic AMP (dBcAMP), a stimulus that induces some features of astrogliosis (Fedoroff et al., 1984; Le Prince et al., 1991). In our analyses, we focused on two factors that are established markers of astrogliosis in several paradigms, basic fibroblast growth factor (FGF-2) and glutamine synthetase (GS) (Eddleston and Mucke, 1993). Observations from neonatal cultures studies were corroborated using an *ex vivo* model of astrogliosis induced in adult animals, an approach demonstrated to retain biochemical changes associated with the reactive phenotype in culture even after multiple divisions (Rozovsky et al., 2005; Wu and Schwartz, 1998).

2. Results

2.1. *In vitro model of astrogliosis*

Neonatal astrocyte cultures were treated with either dBcAMP (1 mM), a synthetic analogue of cAMP, or Aβ peptide 25–35 (Aβ, 25 μM), an aggregating polypeptide implicated in Alzheimer's disease. Exposure to treatments for 48 h induced stellation (Figs. 1A–C) and significantly increased expression of GS (Fig. 1D) and FGF-2 (Fig. 1E). These astrogliosis-related changes were observed as early as 24 h after exposure to dBcAMP and Aβ and persisted for at least 7 days (data not shown).

2.2. *Non-apoptotic activation of caspases in neonatal astrocyte cultures*

To begin evaluating a potential role of caspases in the observed astrogliosis, we assessed caspase activity in astrocyte cultures treated with dBcAMP or Aβ. In comparison to vehicle-treated controls, cultures exposed for 48 h to 1 mM dBcAMP or 25 μM Aβ showed more than a two-fold increase in total caspase activity (Fig. 2A). This increase in caspase activity was apparent within 24 h and persisted for at least 4 days, although there was no significant change in vehicle-treated control cultures (data not shown). The observed elevation in caspase activity was associated with an up-regulation of the cleaved active fragment of caspase-3, the most common effector caspase in the brain (Fig. 2B). Increases in total caspase activity and cleavage of caspase-3 associated with dBcAMP- and Aβ-induced reactivity were modest compared to marked increases observed following treatment of astrocytes with a toxic concentration of staurosporine (1 μM), a broad kinase inhibitor established to induce caspase activation and apoptosis in numerous cell types (Bertrand et al., 1994; Krohn et al., 1998). Importantly, although both 1 mM dBcAMP and 25 μM Aβ increase caspase activity in astrocytes, this action was not associated with changes in cell viability. To ensure that caspase activation was not associated with delayed cell death, we maintained treatment of astrocyte cultures with dBcAMP and Aβ for 4 days before analysis. Neither dBcAMP nor Aβ induced significant cell death compared to vehicle-treated astrocytes, with no differences observed in the number of cells labeled with the cell death marker, ethidium homodimer (Figs. 3A, B) or the release of the enzyme lactate dehydrogenase (Fig. 3C). The proportion of vehicle-