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Age-related severity of focal ischemia in female rats is associated with impaired astrocyte function

Danielle K. Lewis, Kristen T. Thomas, Amutha Selvamani, Farida Sohrabji*

Women's Health in Neuroscience Program, Department of Neuroscience and Experimental Therapeutics, Texas A & M Health Science Center, College Station, TX, USA

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Abstract

In middle-aged female rats, focal ischemia leads to a larger cortical infarction as compared with younger females. To determine if stroke-induced cytotoxicity in middle-aged females was associated with impaired astrocyte function, astrocytes were harvested and cultured from the ischemic cortex of young and middle-aged female rats. Middle-aged astrocytes cleared significantly less glutamate from media as compared with young female astrocytes. Furthermore, astrocyte-conditioned media from middle-aged female astrocytes induced greater migration of peripheral blood monocyte cells (PBMCs) and expressed higher levels of the chemoattractant macrophage inflammatory protein-1 (MIP-1). Middle-aged astrocytes also induced greater migration of neural progenitor cells (NPCs), however, their ability to promote neuronal differentiation of neural progenitor cells was similar to young astrocytes. In males, where cortical infarct volume is similar in young and middle-aged animals, no age-related impairment was observed in astrocyte function. These studies show that the aging astrocyte may directly contribute to infarct severity by inefficient glutamate clearance and enhanced cytokine production and suggest a cellular target for improved stroke therapy among older females.

Keywords: Reproductive senescence; MIP-1; Sex differences; Neuronal differentiation; GLT-1; PBMC

1. Introduction

Stroke is a major cause of mortality and long-term disability and results from a blood vessel occlusion that prevents blood flow, reducing both the amount of oxygen and glucose that can reach the brain. As neural tissue has a high demand for both, this initial event causes a disruption in metabolic processes and leads to a cascade of excitotoxic and inflammatory pathways (for review see Bramlett and Dietrich, 2004; Dirnagl et al., 1999; Hazell, 2007) affecting glutamate regulation (Benveniste et al., 1984; Globus et al., 1988) and increased expression of cytokines and/or chemokines such as nuclear factor-κB (NF-κB), tumor necrosis factor (TNF)-α, hypoxia-inducible factor (HIF)-1, interleukins (Dirnagl et al., 1999), monocyte chemoattractant protein (MCP)-1, and macrophage inflammatory protein (MIP)-1 (Dirnagl et al., 1999; Kim et al., 1995).

Astrocytes play a key role in modulating these early events. Following cerebral ischemia reactive astrocytes are observed at the injury site (Ordy et al., 1993; Petito et al., 1990; Yamamoto et al., 1987) and can provide either trophic support or exacerbate the tissue damage. During pathological glucose shortages, astrocyte-derived glycogen can be degraded to form lactate as an alternative energy source (Brown et al., 2004; Ransom and Fern, 1997; Suh et al., 2007). Astrocytes are also involved in glutamate uptake (Schousboe et al., 1977) through the glial glutamate transporters (glutamate-aspartate transporter; GLAST) which is also known as the excitatory amino acid transporter (EAAT1) (Storck et al., 1992) and the glial glutamate transporter (GLT)-1 also known as the excitatory amino acid transporter-2 (EAAT2) (Pines et al., 1992; for review also

* Corresponding author at: TAM Health Science Center, Department of Neuroscience and Experimental Therapeutics, 228 Reynolds Medical Building, College Station, TX 77843-1114, USA. Tel.: +1 979 845 4072; fax: +1 979 845 0790.

E-mail address: sohrabji@medicine.tamhsc.edu (S. Sohrabji).

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see Amara and Fontana, 2002; Tzingounis and Wadiche, 2007). However, with injury this uptake mechanism can be inhibited (Volterra et al., 1994; Yu et al., 1989). Further, astrocytes are important sources for both inflammatory cytokines/chemokines such as interleukin (IL)-1 and IL-6, TNF-α, interferon (IFN)-γ (Lau and Yu, 2001; Ridet et al., 1997), MCP-1 (Ransohoff et al., 1993), and MIP-1 (Kim et al., 1995) as well as neurotrophins (Schwartz and Nishiyama, 1994; Wu et al., 2004) and growth factors (Garcia-Estrada et al., 1992; Ridet et al., 1997) after injury.

The extent of neural damage may be related to how well astrocytes modulate these injury-induced events, and age-related astrocyte impairment could exacerbate neurodegeneration. In aging rats, where functional recovery is impaired, glial reactivity is accelerated after ischemia (Badan et al., 2003; Popa-Wagner et al., 2007), but this rapid increase drops dramatically 7 days postischemia (Popa-Wagner et al., 2007). Aging astrocytes may also be more sensitive to parenchymal acidosis after ischemia. In aged murine astrocyte cultures, exposure to kainate in combination with Zn^{2+} was only detrimental to astrocyte survival when the pH was lowered (Sensi et al., 2006). Furthermore, the duration of ischemia-induced activation of signal transducers and activators of transcription 3, a factor regulating astrocyte reactivity, was significantly reduced in aged rats as compared with young rats (Dinapoli et al., 2010). Thus, astrocyte activity after injury may be perturbed in aging animals.

Reproductive senescence in rats, which is a midlife event physiologically similar to menopause, may play a strong role in the brain's ability to cope with a neural insult. Our previous studies show reduced neurotrophin expression (Jezierski and Sohrabji, 2001), increased blood brain permeability (Bake and Sohrabji, 2004), and an elevated brain inflammatory response to an excitotoxic injury (Nordell et al., 2003) in this middle-aged group. Furthermore, ischemic injury caused by middle cerebral artery occlusion results in a significantly larger infarct volume in this reproductively senescent, middle-aged group of females as compared with younger females (Selvamani and Sohrabji, 2010a; Selvamani and Sohrabji, 2010b). Astrocytes play a key role in providing trophic support for neurons (Liesi and Silver, 1988; Parish et al., 2002; Ullian et al., 2001; Wujek and Akeson, 1987), are a component of the blood-brain barrier (Janzer and Raff, 1987; Kim et al., 2006; recent review), and regulate the neuroinflammatory response (Fontana et al., 1982; Robbins et al., 1987). Hence we hypothesized that the greater infarct severity seen in middle-aged females is associated with impaired astrocyte function. Additionally, because our studies indicate that cortical infarct volume is not increased in middle-aged males as compared with young males (Supplementary Fig. 1), we hypothesized that the function of ischemia-activated cortical astrocytes would not differ between these 2 ages of male rats. Here we examined the possible role of ischemia-activated cortical astrocytes in reproductively competent female and male rats (young, 6–8 months), reproductively senescent female rats (middle-aged, 10–12 months), and age-matched (reproductively competent) male rats (middle-aged, 10–12 months).

2. Methods

2.1. Animals

Young (6–8 month, average wt: 273.67 ± 17.09 g) and reproductively senescent, middle-aged (10–12 months; average wt: 325.75 ± 11.7 g) Sprague-Dawley female rats were purchased from Harlan Laboratories (Indianapolis, IN, USA). For some studies, age-matched males (young: 6–8 months, average wt: 443.56 ± 45.5 g; middle-aged: 10–12 months; average wt: 498.56 ± 37.52 g) were also used. In females, the estrus cycle was determined by vaginal smears taken each morning for a period of 14–20 days. Vaginal smears were obtained with a cotton swab, placed on a slide and the cytology was examined (Olympus, BX2 microscope, Leeds Precision Instruments, Minneapolis, MN, USA) for estrus cycle classification. In the young cohort, only those females that had a normal estrus cycle were used. Reproductively senescent middle-aged females that persisted in 1 stage for 7 days were considered acyclic and were only used when vaginal smears confirmed that they were in constant diestrus (persistent low estrogen state). All procedures were in accordance with National Institutes of Health (NIH) and institutional guidelines governing animal welfare.

2.2. Generation of adult cortical astrocyte cultures

2.2.1. Intracerebral transient middle cerebral artery occlusion

Cortical astrocytes were collected from deeply anesthetized (ketamine: 87 mg/kg; xylazine: 13 mg/kg) animals that had been subjected to an intracerebral transient middle-cerebral artery occlusion (MCAo) 2 days poststroke. Transient MCAo was performed according to published studies (Biernaskie et al., 2001; Luke et al., 2004) and established laboratory procedures (Selvamani and Sohrabji, 2010a, 2010b). Briefly, animals were deeply anesthetized and placed into a Kopf stereotaxic apparatus. A midline incision was made in the scalp, the skull was exposed and a small hole was drilled on the left side of the skull. Endothelin-1 (ET-1; 3 μL of 0.5 μg/μL, American Peptide Company, Inc., Sunnyvale, CA, USA) was injected adjacent to the middle-cerebral artery (MCA; coordinates: Anterioposterior: +0.9, Mediolateral: +3.4, Dorsoventral: −8.5 relative to bregma) at a rate of 1.0 μL per 30 seconds using a 5 μL Hamilton syringe with a 26s-gauge/2-inch needle. The syringe was slowly removed 3 minutes after the injection to minimize backflow. Rats were maintained at 37 °C throughout the surgical procedures and respiratory rate and oxygen saturation were constantly monitored using the MouseOximeter (STARR Life Sciences Corp., Oakmont, PA, USA) (see Supplementary Table 1). The ET-1 model results in a