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Full-length Article

Hippocampal interleukin-1 mediates stress-enhanced fear learning: A potential role for astrocyte-derived interleukin-1β

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ABSTRACT

Post-traumatic stress disorder (PTSD) is associated with immune dysregulation. We have previously shown that severe stress exposure in a preclinical animal model of the disorder, stress-enhanced fear learning (SEFL), is associated with an increase in hippocampal interleukin-1β (IL-1β) and that blocking central IL-1 after the severe stress prevents the development of SEFL. Here, we tested whether blocking hippocampal IL-1 signaling is sufficient to prevent enhanced fear learning and identified the cellular source of stress-induced IL-1β in this region. Experiment 1 tested whether intra-dorsal hippocampal (DH) infusions of interleukin-1 receptor antagonist (IL-1RA, 1.25 μg per hemisphere) 24 and 48 h after stress exposure prevents the development of enhanced fear learning. Experiment 2 used triple fluorescence immunohistochemistry to examine hippocampal alterations in IL-1β, glial fibrillary acidic protein (GFAP), an astrocyte-specific marker, and ionized calcium binding adaptor molecule -1 (Iba-1), a microglial-specific marker, 48 h after exposure to the severe stressor of the SEFL paradigm. Intra-DH IL-1RA prevented SEFL and stress-induced IL-1β was primarily colocalized with astrocytes in the hippocampus. Further, hippocampal GFAP immunoreactivity was not altered, whereas hippocampal Iba-1 immunoreactivity was significantly attenuated following severe stress. These data suggest that hippocampal IL-1 signaling is critical to the development of SEFL and that astrocytes are a predominant source of stress-induced IL-1β.

1. Introduction

Converging evidence from both human and animal studies has suggested that psychiatric disorders involving depression and anxiety, including post-traumatic stress disorder (PTSD), involve substantial immune system dysregulation (Koo and Duman, 2009; Silverman et al., 2007; Stepanichev et al., 2014; Gill et al., 2009; Jones et al., 2015). Several published studies have reported that PTSD is associated with elevated peripheral cytokines, such as interleukin-1β (IL-β), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) (Gill et al., 2009; Gola et al., 2013; Passos et al., 2015; Guo et al., 2012; Wang and Young, 2016). Cohen and colleagues have even suggested IL-1 as a potential biomarker for susceptibility to PTSD (Cohen et al., 2011). Central IL-1 signaling is consistently shown to be upregulated by a variety of different stress protocols in rodents and to be critically involved in stress response mechanisms that drive behavioral outcomes (Goshen and Yirmiya, 2009; Avital et al., 2003). For example, peripheral administration of IL-1β has been shown to lead to enhanced anxiety-like behavior in the elevated plus maze (EPM) (Swiergiel and Dunn, 2007), and blocking IL-1 signaling centrally prevents stress-induced reductions in social interaction (Arakawa et al., 2009). We recently published the finding that stress-enhanced fear learning (SEFL), a preclinical animal model of PTSD developed by Rau and colleagues (Rau et al., 2005), requires central IL-1 signaling. Our data demonstrated that the severe stressor of the SEFL paradigm (15 foot shocks) induces an increase in IL-1β in the dorsal hippocampus (DH) 24–48 h after the stress. Furthermore, blocking IL-1 signaling in the brain through an intracerebroventricular infusion of IL-1 receptor antagonist (IL-1RA) prevents the development of enhanced fear learning (Jones et al., 2015). Together these data suggest that central IL-1RA may be acting specifically in the hippocampus. Accordingly the first goal of the current study was to test whether hippocampal IL-1 signaling 24–48 h after severe stress is necessary for the expression of SEFL.

The second goal of the current study was to isolate and quantify colocalization of stress-induced IL-1β with cell-specific markers

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glial fibrillary acidic protein (GFAP), ionized calcium binding adaptor molecule-1 (Iba-1), and neuronal nuclear antigen (NeuN) in order to isolate the cellular source of stress-induced hippocampal IL-1β. IL-1β can be expressed by many cell types in the brain, including microglia, astrocytes, and neurons (Yabuuchi et al., 1994; Zhang et al., 2010; Ringwood and Li, 2008; Flannery and Bowie, 2010; Huang et al., 2011; Guasch et al., 2007). A critical component to better understanding the mechanism through which hippocampal IL-1 might influence behavioral outcomes following stress is to identify which cell type(s) produce(s) it in response to stress. While there is evidence of IL-1 expression in neurons (Zhang et al., 2010; Huang et al., 2011; Kwon et al., 2008), there is only one report to our knowledge of an effect of stress on neuron-derived IL-1β. Kwon and colleagues reported an increase in IL-1β colocalized with neuronal nuclei following four days of restraint stress (Kwon et al., 2008). In contrast, there are several published studies to support the potential for microglia-derived or astrocyte-derived IL-1β, as described below.

Microglia are brain macrophage cells that play important roles in the healthy brain, both maintaining the cellular environment and protecting against injury or immune challenge (Mendiola and Cardona, 2017; Block et al., 2007; Minghetti et al., 2005; Perry et al., 1985; Calcia et al., 2016). A substantial population of microglia are present in the hippocampus (Lawson et al., 1990), and IL-1β is just one of the proinflammatory mediators released by activated microglia (Mendiola and Cardona, 2017; Block et al., 2007; Minghetti et al., 2005; Perry et al., 1985). While microglial activation and release of proinflammatory cytokines are well-established in the context of neurodegenerative diseases, there are inconsistencies regarding the timing, brain region–specificity, and direction of the effect of psychological stressors on microglia. Two independent groups reported no change in microglial gene expression in the hippocampus immediately following exposure to foot shock (Brzozowska et al., 2017; Blandino et al., 2009). However, Sugama and colleagues reported that microglial activation was increased one to six hours following a two hour exposure to restraint stress in the thalamus, hypothalamus, and hippocampus (Sugama et al., 2007). Interestingly, in the same report, hypothalamic microglial activation was only associated with an increase in IL-1β mRNA and immunoreactivity when induced by lipopolysaccharide (LPS), but not when induced by restraint stress. Consistent with an increase in microglial activation and proliferation in response to stress, Frank et al. reported that major histocompatibility complex II immunoreactivity (MHC II, predominantly expressed by microglia) was increased in the hippocampus 24 h after inescapable tail shock (Frank et al., 2007), but they observed no change in either GFAP or Iba-1 immunoreactivity in the same tissue.

The final candidate for a potential source of stress-induced IL-1β is astrocytes. Though traditionally viewed merely as neuronal "glue", astrocytes are now known to be critically involved in a diverse array of functions in development and disease (Barres, 2008). Converging evidence from several laboratories using a variety of different severe stress procedures suggests that both GFAP expression and astrocyte process length are altered over time in the brain following stress (Saur et al., 2016; Tynan et al., 2013; Choi et al., 2016; Xia et al., 2013). Choi and colleagues observed an increase in the length and number of astrocyte processes but a decrease in GFAP in the DH one hour, but not 24 h, after exposure to foot shock fear conditioning (Choi et al., 2016). In contrast, others have observed decreases in the number of astrocyte processes either following chronic restraint stress (Tynan et al., 2013) or 24–48 h after foot shock exposure (Saur et al., 2016). Of particular relevance here, Sugama and colleagues found that IL-1β expression was increased specifically in astrocytes, and not microglia, following cold stress (Sugama et al., 2011). The second goal of the current study was to isolate and quantify colocalization of stress-induced IL-1β with GFAP, Iba-1, and NeuN in order to isolate the cellular source of stress-induced hippocampal IL-1β.

Importantly, much of the previous literature regarding gene expression and morphology of both astrocytes and microglia focuses on early time points post-stress. Given that we have previously reported that the IL-1-dependent mechanism that attenuates the development at SEFL is specific to the later time points following stress, 24–48 h (Jones et al., 2015; Szczytkowski-Thomson et al., 2013), here we focus on changes in the DH at 48 h after foot shock stress. Specifically, experiment 1 tested whether IL-1 signaling in the DH is critical to the development of a PTSD-like phenotype in SEFL. Experiment 2 examined stress-induced changes in astrocytes and microglia in the DH and identified the cellular source of stress-induced IL-1β in this region. Analyses from experiment 2 A replicated our previous finding of stress-induced IL-1β in the dorsal hippocampus. Analyses from experiment 2 B quantified GFAP and Iba-1 immunoreactivity to examine stress-induced changes in astrocytes and microglia, respectively. Finally, analyses in experiment 2 C used Bitplane Imaris software in combination with confocal microscopy to visualize the colocalization of IL-1β with GFAP, Iba-1, and NeuN following foot shock to isolate and quantify astrocyte-derived, microglia-derived, and neuron-derived IL-1β, respectively. Collectively, these experiments tested the hypotheses that IL-1 signaling in the DH is critical for the development of SEFL and that astrocytes are the predominant cellular source of hippocampal IL-1β following stress in this context.

2. Methods

2.1. Animals

Male Sprague Dawley rats (225–250 g, Charles River Laboratories, Raleigh, NC) were housed individually under a reversed 12 h light-dark cycle. They were given ad libitum access to food and water and were handled regularly throughout all experiments. All procedures were conducted in accordance with and approval by the UNC Institutional Animal Care and Use Committee.

2.2. Experiment 1: Effect of intra-dorsal hippocampal IL-1RA on SEFL

2.2.1. Surgery

Animals were anesthetized with a 1.0 mg/kg intraperitoneal injection of 9:1 (vol:vol) ketamine hydrochloride (100 mg/ml) mixed with xylazine (100 mg/ml). Guide cannulae (26 Gauge, Plastics One, Roanoke, VA) were directed bilaterally at the DH (AP −3.4 mm, ML ± 3.1 mm, DV −2.2 mm, 15 degrees, relative to bregma). Animals were given one week for postoperative recovery prior to the start of any experimental procedures. Upon completion of the experiment, correct cannula placement was verified and any animals with incorrect placement were dropped from the analysis.

2.2.2. Stress-enhanced fear learning

All animals (N = 36, n = 9) were assigned to a Context A treatment (foot shock or no foot shock) and a drug treatment (IL-1RA or vehicle) and exposed to the SEFL paradigm (Fig. 1), as has been previously described (Jones et al., 2015; Szczytkowski-Thomson et al., 2013). Briefly, on Day 1, animals were exposed to Context A (BRS/LVE, Laurel, MD; H 26.7 cm × D 24.8 cm × W 30.7 cm) which was housed in a separate room with distinct textile, olfactory, and auditory characteristics from the home cage. Animals assigned to the foot shock condition received 15 × 2 mA scrambled foot shocks over 90 min on a 6 min variable interval schedule while control animals were exposed to the context for the same amount of time without foot shocks being delivered. Six days later, animals were exposed to Context B (Med Associates, St. Albans,

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