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Learned helplessness activates hippocampal microglia in rats: A potential target for the antidepressant imipramine

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ABSTRACT

An accumulating body of evidence has demonstrated that inflammation is associated with the pathology of depression. We recently found that psychological stress induces inflammation in the hippocampus of the rat brain through the inflammasome, a component of the innate immune system. Microglia, the resident macrophages in the brain, play a central role in the innate immune system and express inflammasomes; thus, we hypothesized that hippocampal microglia would be key mediators in the development of depression via stress-induced inflammation. To test this hypothesis and to determine how antidepressants modulate microglial function, we used immunohistochemistry to examine the morphological changes that occur in the hippocampal microglia of rats exposed to the learned helplessness (LH) paradigm. We noted significantly increased numbers of activated microglia in the granule cell layer, hilus, CA1, and CA3 regions of the hippocampi of LH rats. Conversely, administering imipramine to LH rats for 7 days produced a significant decrease in the number of activated microglia in the hilus, but not in the other examined regions. Nonetheless, there were no significant differences in the combined number of activated and non-activated microglia either in LH or LH + imipramine rats relative to control rats. In addition, treating the naïve rats with imipramine or fluvoxamine produced no discernible microglial changes. These data suggest that stress activates hippocampal microglia, while certain antidepressants decrease the number of activated microglia in the hilus, but not in other hippocampal regions. Therefore, the hilus represents a candidate target region for the antidepressant imipramine.

1. Introduction

Depression is now widely recognized as an inflammation-related disease, based on extensive data linking depression to a chronic low-grade inflammatory response (Berk et al., 2013). A typical example derived from a clinical setting is that up to 50% of patients treated with interferon-α develop depression-like behavior (Musselman et al., 2001). Interferon-α is a cytokine that is released in the early stage of viral infections, and is a potent stimulator of endogenous pro-inflammatory cytokine release (Capuron and Miller, 2004). Another example is "sickness behavior," which is caused by activation of the peripheral immune system, such as during systemic infections, cancer, or autoimmune diseases, and which results in immune signaling to the brain (Dantzer et al., 2008). In addition, a recent positron emission tomography study reported a depression-associated elevation in prefrontal cortical translocator protein density, which is a marker of neuroinflammation (Setiawan et al., 2015). In support of the clinical reports, preclinical animal studies have demonstrated that activation of the immune system by administering endotoxin (lipopolysaccharide [LPS]) or interleukin-1β (IL-1β), both of which influence hippocampal neurogenesis, induces sickness behaviors that resemble depression (Dunn et al., 2005; Koo and Duman, 2009). Furthermore, it has been shown that psychological stress induces inflammation in the hippocampus of the rat brain through the inflammasome, a component of the innate immune system (Iwata et al., 2016). Altogether, the clinical and preclinical data strongly indicate a relationship between depression and inflammation.

The major cell types associated with inflammation in the brain are astrocytes and, primarily, microglia, which are regarded as the resident immune cells of the brain (Kreutzberg, 1996; Qin et al., 2002). Resting microglial cells respond dynamically to the functional status of synapses (Wake et al., 2009; Wake et al., 2013), with activated microglia producing a variety of noxious substances. These include nitric oxide, free radicals, and cytokines (Vila et al., 2001; Hanisch, 2002; Qin et al., 2002), all of which can precipitate depression (Vavakova et al., 2015). Based on such findings, it is now hypothesized that some forms of depression can be considered as microglial diseases; thus, if impaired microglial function can lead to depression, then these cells represent a therapeutic target for this disease (Yirmiya et al., 2015).

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Indeed, some dysfunctional processes associated with microglia are ameliorated by antidepressants. For instance, one study demonstrated that amitriptyline reduced LPS-stimulated IL-1β release in microglial cultures (Obuchowicz et al., 2006). In addition, the selective serotonin reuptake inhibitors paroxetine and sertraline have been found to inhibit interferon-γ-induced microglial activation (Horikawa et al., 2010). Collectively, these data suggest that microglia play a pivotal role in the pathology of depression.

Here, we hypothesized that hippocampal microglia would be key mediators in the development of depression via stress-induced inflammation. To test this hypothesis and to determine how antidepressants modulate microglial function, we examined the morphological changes that occur in the hippocampal microglia of rats exposed to the learned helplessness (LH) paradigm. In this animal model of depression (Seligman and Beagley, 1975), an animal is initially exposed to uncontrollable stress. When the animal is later placed in a situation where shock is controllable (escapable), the animal has difficulty generating escape responses. This escape deficit is reversed by chronic antidepressant treatment or by the infusion of brain-derived neurotrophic factor into the hippocampus (Shirayama et al., 2002; Iwata et al., 2006). In the present study, we focused on the hippocampus, since we have previously shown that psychological stress induces inflammation in this structure (Iwata et al., 2016). The hippocampus is considered to be a candidate brain region for impaired functions such as decreased neurogenesis that are associated with depression (Duman et al., 1997). For example, it is well-documented that patients with depression show reduced hippocampal volumes (Sheline et al., 1996; Bremner et al., 2000).

In the present study, we showed that the LH paradigm increased the number of activated microglia in the granule cell layer (GCL), hilus, CA1, and CA3 region of the rat hippocampus. This change was reversed in the hilus only by subchronic administration of imipramine. Subchronic administration of imipramine or fluvoxamine to naïve rats produced no microglial changes. These results indicate that stress activates hippocampal microglia, and imipramine reversed this activation to resting state in the hilus only. This supports our hypothesis that hippocampal microglia are a candidate target for the antidepressant imipramine.

2. Materials and methods

2.1. Animals and treatments

Procedures involving animals were conducted in accordance with the Tottori University Guide for the Care and Use of Laboratory Animals, and the study was approved by the Tottori University Animal Care and Use Committee. Male Sprague Dawley rats (200–230 g) were used in all experiments and housed under a 12-h light/dark cycle, with free access to food and water.

2.2. Experimental design

Three experimental procedures were performed. First, rats were sacrificed 2 days after the acquisition of LH (experiment 1, Fig. 1A). In the second, LH rats were sacrificed 24 h after 7 days of subchronic treatment with either imipramine or a saline control (experiment 2, Fig. 1B). In the third, naïve rats were sacrificed 24 h after 7 days of subchronic treatment with imipramine or fluvoxamine (experiment 3, Fig. 1C).

2.3. Learned helplessness paradigm

LH behavioral tests were performed as described previously (Iwata et al., 2006; Iwata et al., 2011) using the Gemini Avoidance System (San Diego, CA, USA). This apparatus consists of two compartments, which were divided by a retractable door. On days 1 and 2, rats were subjected to 60 inescapable electric foot shocks (0.65 mA, 20–40 s in duration, mean: 30 s). On day 3, a two-way conditioned avoidance test

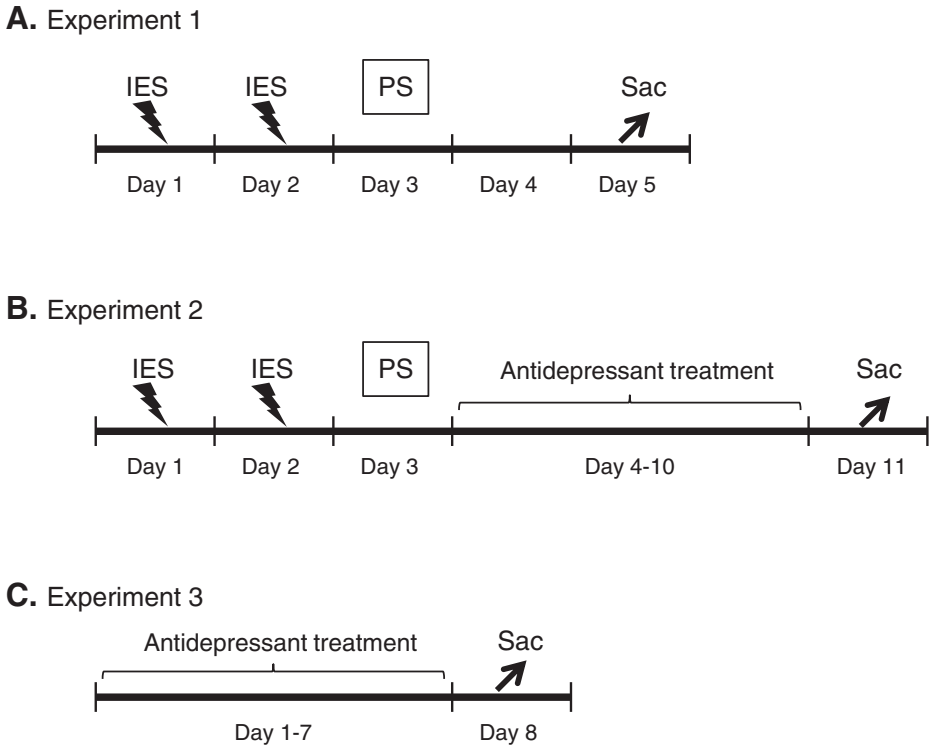

Fig. 1. Experimental procedures. (A) The experimental paradigm for learned helplessness (LH). Rats were subjected to inescapable electric foot shocks for 2 days, followed by two-way conditioned avoidance testing; rats were sacrificed two days after the acquisition of LH. (B) Experimental paradigm for LH + imipramine treatment. Rats were subjected to inescapable electric foot shocks for 2 days, followed by two-way conditioned avoidance testing; rats were sacrificed 24 h after subchronic treatment with either imipramine or saline for 7 days. (C) The experimental paradigm for antidepressant treatments. Rats were subjected to 7 days of subchronic treatment with imipramine, fluvoxamine, or saline, and sacrificed 24 h later. IES: inescapable electric foot shocks, PS: postshock test, Sac: sacrifice.