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Full-length Article

The protective effects of resveratrol on social stress-induced cytokine release and depressive-like behavior

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ABSTRACT

Social stress is a risk factor for psychiatric disorders, however only a subset of the population is susceptible while others remain resilient. Inflammation has been linked to the pathogenesis of psychosocial disorders in humans and may underlie these individual differences. Using a resident-intruder paradigm capable of revealing individual differences in coping behavior and inflammatory responses, the present study determined if resveratrol (RSV; 0, 10, 30 mg/kg/day) protected against persistent stress-induced inflammation in socially defeated rats. Furthermore, the antidepressant efficacy of RSV was evaluated using the sucrose preference test. Active coping rats were characterized by more time spent in upright postures and increased defeat latencies versus passive coping rats. Five days after defeat, flow cytometry revealed enhanced stimulation of proinflammatory proteins (IL-β, TNF-α) in spleen cells of passive rats as compared to active coping and controls, an effect that was blocked by both doses of RSV. Furthermore, only passive coping rats exhibited increased proinflammatory proteins (IL-1β, TNF-α, GM-CSF) in the locus coeruleus (LC), a noradrenergic brain region implicated in depression. Notably, only 30 mg/kg RSV blocked LC neuroinflammation and importantly, was the only dose that blocked anhedonia. Alternatively, while stress had minimal impact on resting cytokines in the dorsal raphe (DR), RSV dose-dependently reduced DR cytokine expression. However, this did not result in changes in indoleamine 2,3-dioxygenase activity or serotonin levels. Taken together, these data suggest that social stress-induced depressive-like behavior evident in passive coping rats may be driven by stress-induced neuroinflammation and highlight natural anti-inflammatory agents to protect against social stress-related consequences.

1. Introduction

It has long been recognized that repeated exposure to social stress can result in the development of depression (Almeida, 2005). Affecting approximately 7 percent of adults and 11 percent of adolescents, depression is considered to be one of the most common debilitating diseases in the United States (National institute of mental health 2013a,b), yet there is significant individual variability in susceptibility, which has been attributed to differences in coping style (Veenema et al., 2003). In humans, passive coping, such as avoidance or substance abuse, is associated with increased stress susceptibility, while active coping, such as problem solving, is associated with stress resiliency (Cairns et al., 2014). This phenomenon has also been observed in rodents where passive coping results in the development of physiological and behavioral endpoints comparable to a depressive-like state, which is not evident in animals which adopt active coping strategies (Ahmed et al., 2014; De Miguel et al., 2011; Gomez-Lazaro et al., 2011; Korte et al., 1992; Perez-Tejada et al., 2013; Wood et al., 2010; Wood et al., 2015, 2013).

Interestingly, coping in both humans and rodents has been shown to play a large role in inflammatory responses to an immune challenge. For example, lipopolysaccharide stimulation of cytokine release in whole plasma is greater in passive versus active coping individuals (Bouhuys et al., 2004). Furthermore, unstimulated resting levels of peripheral cytokines, such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α are increased in subpopulations of depressed patients (Alesci et al., 2005; Bouhuys et al., 2004; Maes et al., 1997; Miller et al., 2002; Motivala et al., 2005; Musselman et al., 2001; Raison et al., 2013). Although much of what is known about depression and

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inflammation is obtained from plasma measurements, recent studies detected evidence of increased neuroinflammation during major depressive episodes regardless of peripheral inflammatory status (Setiawan et al., 2015). Data from our lab and others have revealed that social stress can produce increased inflammation both peripherally and centrally and that this increased inflammatory response occurs only in rats that are susceptible to social defeat-induced depressive-like behaviors (Hodes et al., 2014; Li et al., 2015; Wood et al., 2015). As a result, social defeat stress represents a useful tool for identifying how neuroinflammation contributes to the pathophysiology of these stress-related disorders.

Historically depression has been associated with dysfunctions of both the noradrenergic (NE) and serotonergic (5-HT) systems. This is believed to occur through dysregulation of the locus coeruleus (LC), the major source of NE to the brain (Aston-Jones et al., 1995; Ressler and Nemeroff, 2000; Swanson and Hartman, 1976), and the dorsal raphe (DR), a major source of 5-HT (Lechin et al., 2006; Roche et al., 2003). While it is well recognized that these systems may play a significant role in the pathophysiology of depression, the mechanistic basis by which this occurs is still unclear. Interestingly, inflammation has been shown to affect both of these systems; inflammatory cytokines increase the spontaneous firing rate of the LC (Borsody and Weiss, 2002, 2004) and within the DR they promote serotonergic cell death and a shift away from 5-HT synthesis by activation of the indoleamine 2,3-dioxygenase (IDO) enzymatic pathway (Guillemin et al., 2003; Hochstrasser et al., 2011; Lestage et al., 2002). Likely due, in part, to increased inflammatory responses, enhanced NE tone throughout the brain (Page and Abercrombie, 1999), reduced 5-HT, and elevated kynurenine (Kyn), a neurotoxic byproduct of the IDO pathway, have been associated with depression (Guillemin et al., 2003; Hochstrasser et al., 2011; Lestage et al., 2002). Therefore, this study focused on the contribution of stress-induced neuroinflammation within the LC and DR in the development of depressive-like behaviors.

Given the role that inflammation may play in depressive disorders, anti-inflammatory agents may prove useful as an antidepressant therapy or adjuvant to traditional therapies. Resveratrol (RSV) is a natural, commercially available polyphenol found in the skin of red grapes and red wine (Langcake and Pryce, 1976). RSV has demonstrated anti-inflammatory properties (Donnelly et al., 2004; Falchetti et al., 2001; Fordham et al., 2014) through inhibition of mast cell, macrophage, neutrophil, and microglial production of histamines, cytokines, proteases, nuclear factor-kappa B, and oxidants (de la Lastra and Villegas, 2005; Zhang et al., 2010). These cellular effects are thought to be responsible for RSV's anxiolytic properties (Patki et al., 2013a) as well as its demonstrated anti-depressant efficacy in the forced swim test (Ahmed et al., 2014; Xu et al., 2010), single prolonged stress (Solanki et al., 2015), and Wistar-Kyoto depressive-like rat model (Hurley et al., 2014). The aim of the present study was to test the ability of RSV to suppress stress-induced inflammation resulting from exposure to an ethologically relevant model of social stress in rodents. Furthermore, these studies differentiated between the contribution of central (LC/DR) and peripheral inflammation in the development of a depressive-like phenotype. In addition to measuring neuroinflammation in the LC, these studies quantified the effects of stress and RSV on inflammation and IDO activity within the serotonergic DR.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (225–250 g, intruder or controls) and Long-Evans retired breeders (650–850 g, residents) (Charles River, Wilmington MA) were individually housed in standard cages with ad libitum access to food and water while maintaining a 12-h light/dark cycle. Care and use of the animals was approved by the University of South Carolina's IACUC and was in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Resveratrol treatment

Trans-resveratrol (Cayman Chemical Company, Ann Arbor, MI) was dissolved in a solution consisting of 10% ethanol (Ultra Pure, Darien, CT), 15% Cremophor (Fisher Scientific, Waltham, MA), and 75% saline (Abbott Laboratories, Chicago, IL) to a concentration of 10 or 30 mg/ml. These doses were chosen as 10 and 30 mg/kg RSV treatment produce peripheral anti-inflammatory effects (Hong et al., 2008; Singleton et al., 2010), yet only the higher dose of 30 mg/kg accesses the brain as identified by high performance liquid chromatography studies (Wang et al., 2002). Therefore, vehicle (0), 10, or 30 mg/kg RSV (ip) was administered to rats beginning 7 days before control/defeat exposure and ending on the last day of defeat. All tissue was collected 5 days after the final treatment with RSV (see Fig. 1 for a brief study timeline). Since the half-life of RSV following repeated dosing is reported to be 2.5 h, RSV is not likely to persist in plasma or brain tissue at time of collection (Almeida et al., 2009).

2.3. Social stress (resident-intruder paradigm)

This animal model is modified from the version developed by Miczek (1979) and identical to our previous publications (Wood et al., 2010, 2015, 2013). Long-Evans retired breeders were screened for their level of aggression prior to being included in the study. Inclusion criteria consisted of 1) exhibiting an attack latency of less than 60 s, 2) total number of attacks $\geqslant 4$ within the first 5 min, and 3) effective attacks that did not result in injury to the intruder. Sprague-Dawley rats were randomly assigned into the "intruder" or "control" group. Intruders were exposed to a different Long-Evans retired breeder for 30 min for five consecutive days and behavioral responses of the resident and intruder were noted in addition to defeat latency. After exhibiting a supine posture, or after 15 min, whichever came first, intruders were placed behind a Plexiglas partition within the resident's cage for the remainder of the 30-min defeat period. Each day following social defeat intruders were returned to their home cage. Control animals were not present in the room during social defeat exposures and control manipulation consisted of being placed into a novel cage behind a partition for 30 min/day. Defeat exposures were video recorded for a subset of rats from each treatment group. An experimenter blinded to the treatment conditions quantified the duration of time each intruder spent in an upright posture upon the 1st and 5th day of social defeat as previously reported (Wood et al., 2010).

2.4. Sucrose preference

All experimental animals were subject to the sucrose preference test 2–3 days prior to stress/control exposure and 4 days after the final stress/control exposure as previously published (Wood et al., 2015). Sucrose preference ([volume 1% sucrose/total volume consumed] $\times$ 100) was calculated for the first hour of the dark period.

2.5. Tissue collection

Trunk blood, brains, and spleens were collected upon time of sacrifice 5 days after the final defeat or control exposure. Brains were flash frozen in isopentane, and stored at -80 °C. The posterior brain was sliced coronally using a cryostat up to the most caudal