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Imipramine attenuates neuroinflammatory signaling and reverses stress-induced social avoidance

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ABSTRACT

Psychosocial stress is associated with altered immunity, anxiety and depression. Previously we showed that repeated social defeat (RSD) promoted microglia activation and social avoidance behavior that persisted for 24 days after cessation of RSD. The aim of the present study was to determine if imipramine (a tricyclic antidepressant) would reverse RSD-induced social avoidance and ameliorate neuroinflammatory responses. To test this, C57BL/6 mice were divided into treatment groups. One group from RSD and controls received daily injections of imipramine for 24 days, following 6 cycles of RSD. Two other groups were treated with saline. RSD mice spent significantly less time in the interaction zone when an aggressor was present in the cage. Administration of imipramine reversed social avoidance behavior, significantly increasing the interaction time, so that it was similar to that of control mice. Moreover, 24 days of imipramine treatment in RSD mice significantly decreased stress-induced mRNA levels for IL-6 in brain microglia. Following *ex vivo* LPS stimulation, microglia from mice exposed to RSD, had higher mRNA expression of IL-6, TNF-α, and IL-1β, and this was reversed by imipramine treatment. In a second experiment, imipramine was added to drinking water confirming the reversal of social avoidant behavior and decrease in mRNA expression of IL-6 in microglia. These data suggest that the antidepressant imipramine may exert its effect, in part, by down-regulating microglial activation.

1. Introduction

Psychosocial stress stimulates the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic nervous system (SNS), triggering the release of catecholamines, glucocorticoids and pro-inflammatory cytokines such as interleukin (IL)-6, (IL)-1, and tumor necrosis factor (TNF)-α. Activation of neuroendocrine and autonomic pathways has a profound impact in physiological responses in both humans and rodents (Blanchard et al., 2001; Kiecolt-Glaser and Glaser, 2002; Kinsey et al., 2007). Converging translational evidence suggests that psychosocial stress-induced, peripheral immune dysregulation and neuroinflammation, contribute to the development of depressive-like and anxiety-like behaviors (Voorhees et al., 2013; Wohleb et al., 2011, 2013, 2014). Pro-inflammatory cytokines such as IL-6, IL-1β, and TNF-α modulate neuronal activity (Ozaktay et al., 2006). Likely, stress-induced neuroinflammatory signaling increases neuroplasticity that can lead to modification in the connectivity between neurons and neuronal circuits underlying behavioral disorders such as prolonged anxiety and depressive symptoms (Koo and Duman, 2008; Koo et al., 2010; Elliott et al., 2010; Christoffel et al., 2011).

The clinically relevant psychosocial stress model of repeated social defeat (RSD), promotes brain region-specific activation of brain $CD11b^+$ cells (microglia/macrophages) that leads to anxiety-like behavior. In addition, microglia isolated from socially defeated mice have high levels of IL1-β mRNA expression and reduced levels of glucocorticoid responsive genes (glucocorticoid-induced leucine zipper (GILZ) and FK506 binding protein-51 (FKBP51) (Wohleb et al., 2011). Furthermore, microglia isolated from these mice and cultured *ex vivo* produced increased levels of IL-6, TNF-α, and monocyte chemoattractant protein-1 (MCP-1/CCL-2) following mitogen-stimulation with lipopolysaccharide (LPS) compared to microglia from home cage controls (Wohleb et al., 2011). RSD enhances reactivity of microglia and

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macrophages in a brain-dependent manner. In a previous study we determined reactivity of microglia and macrophages through Iba-1 staining in the medial amygdala, pre-frontal cortex (PFC), and paraventricular nucleus of the hypothalamus, after 6 days of RSD. These findings showed that social defeat enhanced the active microglia phenotype in several areas of the brain associated with fear and threat appraisal, after 6 days of RSD (Wohleb et al., 2011). Iba-1 labeling of microglia and increased Iba-1 proportional area was detected in the PFC at .5, 8, and 24 days after RSD. Additionally, immunoreactivity was also detected in the amygdala, hippocampus (HPC)-cornu ammonis 3 (CA3), and HPC-dentate gyrus at .5 and 8 days after RSD, but no longer was detected by 24 days. These data suggest that microglia return to a surveying state after RSD, in a time-dependent manner (Wohleb et al., 2014).

Clinical and experimental approaches indicate that antidepressants attenuate brain expression of pro-inflammatory cytokines and evoke neuroprotective and immunomodulatory effects (Sluzewska et al., 1995; Xia et al., 1996; Yirmiya et al., 2001; Castanon et al., 2002; Hashioka et al., 2007; Hwang et al., 2008). In clinical studies, the therapeutic effects of antidepressants seem to be related to the immune status of depressed patients when treatment is initiated. For example, when depressed patients had enhanced immune activation, antidepressants attenuated secretion of cytokines. Elevated plasma levels of IL-6 in patients suffering from acute depression were reduced when these patients were treated with fluoxetine, a selective serotonin reuptake inhibitor (Sluzewska et al., 1995). Antidepressants also normalized increased counts of monocytes, leukocytes and neutrophils in depressed patients (Seidel et al., 1996; Maes et al., 1997). Conversely, when immunity was not altered in depressed patients at the initiation of treatment, antidepressants had no effects on immune function.

In animal models, imipramine (a tricyclic antidepressant) and fluoxetine produce immune suppression and anti-inflammatory effects by suppressing the production of cytokines such as TNF-α, IL-1β, and IL-6 by glial cells (Ha et al., 2006; Lim et al., 2009; Liu et al., 2011). Imipramine inhibited interferon (IFN)-γ stimulated microglial production of IL-6 and nitric oxide (Hashioka et al., 2007), and TNF-α production in microglia and astrocyte cultures (Hwang et al., 2008). In addition to the effects on immune function, antidepressants can also modulate behavior. Specifically, imipramine treatment ameliorated LPS-induced depressive-like behavior in rats, decreased anhedonia, anorexia, weight loss, reduced social, locomotor, and exploratory behaviors (Yirmiya, 1996; Yirmiya et al., 2001). In mice subjected to social stress, 28 days of chronic administration of fluoxetine or imipramine, but not acute administration (1 day), improved social interaction in the social avoidance behavioral test (Berton et al., 2006; Tsankova et al., 2006).

Recent findings from our laboratory showed that RSD promotes long-lasting microglial activation associated with social avoidance behavior, which is maintained for at least 24 days after cessation of RSD (Wohleb et al., 2014). Thus, we aimed to determine: (1) if imipramine treatment reversed RSD-induced social avoidance behavior and (2) if the stress-induced neuroinflammatory profile, maintained at 24 days after RSD, was attenuated with imipramine treatment.

2. Materials and methods

2.1. Animals

Male C57BL/6 (6–8 weeks old) and CD-1 (12 months, retired breeders) mice were obtained from Charles River Breeding Laboratories (Wilmington, Massachusetts) and allowed to acclimate to their surroundings for 7–10 days before initiation of experimental procedures. C57BL/6 mice were housed in cohorts of three and CD-1 mice were singly housed and maintained at 21 °C under a 12:12 h light: dark cycle with *ad libitum* access to water and rodent chow in the animal facility at The Ohio State University. All procedures were in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and were approved by the Ohio State University Institutional Laboratory Animal Care and Use Committee.

2.2. Repeated social defeat (RSD)

RSD was performed as described previously (Wohleb et al., 2011). Briefly, an intruder male CD-1 mouse was introduced into home cages of male C57BL/6 mice (three per cage) for 2 h on 6 consecutive nights. Behavior was observed to make certain that the intruder was aggressive. If the intruder did not initiate an attack within 5–10 min or was attacked by resident mice, a new intruder was introduced. At the end of the 2 h the intruder was removed and the resident mice were left undisturbed until the next day when the same paradigm was repeated. During RSD, resident mice display submissive behaviors such as upright posture, fleeing, and crouching (Avitsur, 2001; Stark et al., 2001; Hanke et al., 2012). Home cage control (HCC) cohorts were left undisturbed in a separate room.

2.3. Pharmacological treatments and administration procedures

C57BL/6 mice subjected to RSD and HCC were randomly selected for inclusion into different experimental treatment groups. The groups were: RSD/imipramine, RSD/vehicle, HCC/imipramine, and HCC/vehicle. Mice in the RSD/imipramine received daily intraperitoneal (i.p.) injections of imipramine (20 mg/kg) for 24 days after the 6 cycles of RSD. HCC/imipramine received daily i.p. imipramine at the same dose while RSD/vehicle and HCC/vehicle groups received i.p. injections of vehicle (sodium chloride, 0.9%) for 24 days at the same time point (Fig. 1A). This dose and timing was chosen since previous studies had shown that chronic (4 weeks) but not acute (1 day) imipramine treatment after social defeat, at this concentration, reversed social avoidance behavior in C57BL/6 mice (Berton et al., 2006; Tsankova et al., 2006). Additionally, we had previously shown social avoidance behavior was still present 24 days after RSD (Wohleb et al., 2014). Therefore, based on these studies, we decided to treat the animals for 24 days after the last cycle of RSD to assess if there was a reversal of social avoidance. The day after the last injection of imipramine the interaction and avoidance toward an unfamiliar CD-1 mouse was measured.

2.4. Social avoidance test

Social avoidance behavior was determined as previously described (Wohleb et al., 2014). In brief, the social avoidance test consists of two trials. In the first trial, an experimental mouse was placed into the arena with an empty wire mesh cage and activity was recorded for 2.5 min. In the second trial, the experimental mouse was placed in the arena with an unfamiliar CD-1 mouse in the wire mesh cage and activity was recorded for the same amount of time. Time in the interaction zone and time spent in the corners was video-recorded and analyzed using Noldus EthoVision Software (Leesburg, Virginia) (n = 12–15 per group).

2.5. Splenocyte isolation and culture conditions

Spleens from experimental and control mice were aseptically removed and mechanically disrupted in 5 ml of ice-cold Hanks balanced salt solution (HBSS) using a Model 80 Biomaster Lab System Stomacher (Seward, Riverview, FL) as previously described (Avitsur, 2001 and Stark et al., 2001). The homogenized solution