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Research report

Corticosterone and immune cytokine characterization following environmental manipulation in female WKY rats

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HIGHLIGHTS

- Female WKY rats had significantly higher post-stress corticosterone.
- IL-1β was abnormally low in WKY females.
- There is a significant decrease in TNF-α following enrichment in the Wistar strain.
- WKY rats in isolation tended to have the lowest corticosterone levels.

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ABSTRACT

This study investigated the effects of environmental manipulation on female Wistar Kyoto (WKY), an animal model of depression, and female Wistar rats. It explored the function of the hypothalamic-pituitary-adrenal axis (HPA) and immune system, as they have both been implicated in the pathophysiology of depression. A further goal was to characterize the immune cytokine concentrations of female WKY rats as this has, to our knowledge, never been documented. Animals were assigned to enriched, standard, or impoverished housing for four consecutive weeks. Following this, serum was collected at baseline and post-stress periods to measure the concentration of corticosterone, TNF-α, IL-1β, and IL-10. WKY animals had significantly higher corticosterone levels at the post-stress time-point than their Wistar counterparts. WKY females in isolation tended to have the lowest corticosterone levels which may indicate that they prefer a solitary environment, a symptom of depression. We observed a significant decrease in TNF-α after enrichment in the Wistar strain. A similar decrease in TNF-α was found in the WKY strain, but there was no difference between environmental conditions. There was a significant increase in pre- to post-stress IL-10 level in both Wistar and WKY animals. WKY females had a significantly lower level of IL-1β as compared to the Wistar animals at both pre- and post-stress time points. Given this strain difference, it is likely that the WKY rats had a dysregulated HPA axis which further influenced their circulating cytokine levels. Further studies are needed to examine how this pattern of findings plays a role in the pathophysiology of depression.

1. Introduction

Depression is a serious disorder with a large impact on both an individual's quality of life and society as a whole. The behavioural symptoms include anhedonia, fatigue, changes in sleep, and withdrawal from social situations [1]. However, the pathophysiological changes in the brain which occur during depression are still not well understood. For instance, some of the issues that have yet to be resolved are related to the slow onset of the therapeutic response of antidepressants and their clinical efficacy in only a subset of patients [2]. Moreover, the scientific evidence linking a chemical imbalance in the brain and depression has recently been called into question [3]. The view that depression is a chemical imbalance in the brain is likely one piece of a much more complex puzzle and other mechanisms and their interaction in the pathophysiology of depression should be explored.

Chronic stress has long been linked to depression, typically through the dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis [4]. One frequently studied marker of stress is the amount of circulating cortisol. Atypical diurnal and reactive stress patterns can reveal an impairment of the HPA axis. For example, an increase in serum cortisol, known as hypercortisolemia, is reported in patients with depression [5,6]. However, the mechanism behind this HPA dysregulation leading to elevated circulating

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cortisol is still unknown [7]. A decrease in serum cortisol, known as hypocortisolism, is also common in patients with major depressive disorder [8]. The findings that both too much and too little circulating cortisol are reported in depression illustrates the complicated mechanisms that underlie mood disorders and the need for more studies.

More recently, a role for the immune system in the pathophysiology of major depression has been uncovered [9]. In response to infection, peripherally produced pro- and anti-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin 10 (IL-10) respectively are released. These pass though the blood-brain barrier and directly affect neural structures [10]. For instance, pro-inflammatory cytokines can increase excitotoxicity and decrease monoamines and trophic factors in the brain [9]. Not only are serum pro-inflammatory cytokine levels higher in people with depression, but there appears to be an imbalance between the pro- and anti-inflammatory cytokine system [11]. The above suggest that a dysregulation in the immune system and the resulting elevation in pro-inflammatory cytokines may predispose an individual to developing depression.

One way to characterize depression-related immune mechanisms is via animal models. For example, rats treated with lipopolysaccharide (LPS), the major component of gram-negative bacteria [12] exhibit sickness behaviour which has similarities to the symptoms of depression including fatigue, anhedonia, pain, and both social and physical withdrawal [13,14]. It has been hypothesized that 'depression' or 'sickness behaviour' has evolved as a motivational state in response to infection as it promotes immune system activation and compensatory energy saving mechanisms [15–17]. The connection between depression and sickness behaviour is strengthened through studies showing that chronic treatment with anti-depressants can alleviate sickness behaviour in rodents [18–20]. In fact, if an increase in cytokines is chemically blocked with anti-inflammatories such as dexamethasone, no sickness behaviour is exhibited since there is no triggering signal [17,21]. While sickness behaviour can be adaptive in response to infection, it can be the cause of psychopathology when it, or its antecedents, are not deactivated [13].

While infection can potently activate the immune system, research has shown that stressful stimuli can also trigger the immune response. Chronic stress early in life can affect inflammatory responses and again contribute to a dysregulation of the HPA axis even in medically healthy patients [22] without exposure to infection. It is known, via a feedback mechanism, that stress-induced inflammatory responses increase circulating cortisol through HPA axis stimulation [9]. Taken together, there is powerful evidence that stress-induced immune activation and its effects on the HPA axis play a large role in the pathophysiology of anxiety and depression.

Physical exercise and social support have been found to help alleviate mild to moderate depression in humans and have been successfully used as interventions [23,24]. However, it is not clear whether social and physical interventions are helpful in cases of severe depression and how this would affect circulating stress hormone and immune cytokine levels. One way to examine this relationship in animal models is by incorporating environmental enrichment (EE) as a form of physical and social enrichment. While EE currently has no standardized format, it typically involves exposing laboratory animals to physical and social stimuli that they would not normally experience in a standard housing condition [25]. EE has been shown to significantly increase neurogenesis as well as decrease anxiety- and depressive-like behaviour [26]. EE can affect neurotransmission [27] as well as increase neurotrophic factors in the hypothalamus of female rats [28]. Furthermore, environmental enrichment, when coupled with brain stimulation reward, has been shown to buffer the effects of LPS injection and the consequent sickness behaviour [14].

Animal models of depression have been used as research tools to study the factors and mechanisms underlying depression. For example, the Wistar Kyoto strain specifically has been utilized as an animal model of depression for the past two decades [29]. This strain was originally bred to be the normotensive control of the spontaneously hypertensive rat [30]. They exhibit depressive-like behaviour in the forced swim test [31], have abnormal circadian rhythm activity [32], are more prone to stress-induced ulcers [33], and are considered the most likely candidates as an animal model of antidepressant-resistant depression [34,35]. While many diseases are more prevalent in women, most animal studies have relied on studies of male animals (for review of sex bias in animal studies see [36,37]). This is also the case in depression and anxiety research. While women have almost twice the likelihood of developing depression in their lifetime, pre-clinical studies of depression have largely employed male animals [37,38]. This bias is due to the hormonal fluctuations inherent in female animals during the estrous cycle which can affect both physiology and behaviour [39]. In recent years, this issue has been gaining more traction and concrete steps are being taken to balance the use of female and male animals although there is still much work to be done [40].

The study described here addressed whether environmental manipulation in the form of enrichment or impoverishment affects the peripheral concentration of corticosterone and the immune cytokines TNF-α, IL-1β, and IL-10 in the WKY strain as compared to that of the Wistar strain. Due to the dearth of studies investigating female depressive responses, particularly in the WKY strain, it is the wider objective of this study to produce data that can be used as a reference for future studies of these strains.

2. Methods

2.1. Animals and environmental conditions

A total of 36 WKY and 36 Wistar female rats at age post-natal day (PND) 28 were obtained from a local supplier (Charles River Laboratories, Québec, Canada). Fig. 1 shows the timeline of the study. At PND 28 rats arrived at the facility and were placed in groups of 3 and left to acclimate for 4 days. Following this, animals were handled between PND 32 and PND 62 and behavioural testing as described in Mileva et al., 2015 was carried out. A day/night cycle of 12 h:12 h and temperature of 21.5 ± 1 °C with humidity ~40% kept conditions standardized. Animals were cycling at PND 55 at which age they are considered sexually mature [41]. At PND 62, they were randomly assigned to one of three environments for 30 days: 1) Standard housing (SH) – 3 rats in one guinea pig cage, with dimensions 50 cm x 38 cm x 20 cm, 2) Isolated housing (IH) – 1 rat per small cage, dimensions 45 cm x 22 cm x 20 cm, or 3) Environmental enrichment (EE) – 6 animals in a multistory cage with toys, chain bridges, Nestlets®, wooden chewing blocks, multicoloured Plexiglas® houses, and cardboard tubes. Animals in EE also had unlimited access to a running wheel. In each environment, food and water was provided ad libitum and cages were cleaned once a week to provide fresh bedding. Each environmental condition had twelve animals. Therefore, 12 WKY animals and 12 Wistar animals were in the EE condition, another 12 WKY and 12 Wistar animals in the SH condition, and a final 12 WKY and 12 Wistar animals in the IH condition.

2.2. Estrous cycle monitoring

Details of estrous cycle stage monitoring are detailed in Mileva et al., 2015. Briefly, vaginal fluid was collected by inserting and