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Research report

Differential visceral nociceptive, behavioural and neurochemical responses to an immune challenge in the stress-sensitive Wistar Kyoto rat strain

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HIGHLIGHTS

- The genetically stressed WKY rat mounts a similar peripheral immune response to LPS as SD rats.
- This strain does not display the same sickness behaviours or pain response to an immune challenge as SD rats.
- Heightened stress of the WKY rats may account for differences in immune-responsive brain substrates of sickness behaviour.

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ABSTRACT

A highly regulated crosstalk exists between the immune and neuroendocrine systems with the altered immune responses in stress-related disorders being a valid example of this interaction. The Wister Kyoto (WKY) rat is an animal model with a genetic predisposition towards an exaggerated stress response and is used to study disorders such as depression and irritable bowel syndrome (IBS), where stress plays a substantial role. The impact of a lipopolysaccride (LPS) immune challenge has not yet been investigated in this animal model to date. Hence our aim was to assess if the stress susceptible genetic background of the WKY rat was associated with a differential response to an acute immune challenge. Central and peripheral parameters previously shown to be altered by LPS administration were assessed. Under baseline conditions, WKY rats displayed visceral hypersensitivity compared to Sprague Dawley (SD) control rats. However, only SD rats showed an increase in visceral sensitivity following endotoxin administration. The peripheral immune response to the LPS was similar in both strains whilst the central neurochemistry was blunted in the WKY rats. Sickness behaviour was also abrogated in the WKY rats. Taken together, these data indicate that the genetic background of the WKY rat mitigates the response to infection centrally, but not peripherally. This implies that heightened stress-susceptibility in vulnerable populations may compromise the coordinated CNS response to peripheral immune activation.

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1. Introduction

The Wistar Kyoto (WKY) rat is frequently employed as a preclinical model of stress related gastrointestinal (GI) disorders [1–5] and depression [6–8] and shows increased activation of central areas associated with emotional aspects of pain perception during colorectal distension (CRD) [9]. Behaviourally these rats are more sensitive to chronic stress than Sprague Dawley rats and show increased depressive behaviour [10,11]. Increased behavioural responses to CRD has been reported [2,12,13], coupled with alterations in colonic accommodation [14,15], and increased defecation [1]. WKY rats also exhibit altered somatic nociceptive response [16] as well an altered hormonal activity and circadian rhythm [17]. In

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addition, there are regional differences in central monoaminergic levels between WKY and SD strains of rat at baseline which reflects their behaviour [18].

Inappropriate immune responses have been implicated in stress-related disorders [19–21] [22,23]. The influence of the stress driven neuroendocrine system on immunity is mediated by a number of factors including glucocorticoids, catecholamines, endogenous opioids as well as pituitary hormones such as growth hormone [24,25].

Experimental administration of lipopolysaccharide (LPS), a cell wall component of Gram negative bacteria, is an established model of a transient systemic immune activation that causes a variety of effects including fever [26], sickness behaviour [27] and hyperalgesia [28,29]. These responses, in many cases, requires the synthesis, secretion and action of cytokines [30]. The primary cytokines involved are interleukin (IL)-1, tumour necrosis factor (TNF)-α and IL-6 [27]. Initially produced locally, these cytokines have been implicated in the central components of the host response to infection [31]. Sickness behaviour symptoms along with fever constitute an organised and evolutionary conserved approach by the organism to fight infection [27,32,33]. Animals that are not able to mount the appropriate immune response following an infection show lower survival rates than those showing an activated immune response [34].

One of the manifestations of immune activation is visceral sensitivity [35,36]. Processing of nociceptive signals from the viscera involves an integrated network of central structures including the sensory cortex, thalamus, hypothalamus and the midbrain. These central brain areas modulate nociceptive signals at the spinal cord level via descending pathways [37].

Although the WKY has been shown to have endocrine, molecular, neurochemical differences compared with control SD rats it is currently unclear if the response to an immune challenge is altered. Thus an alteration in such a response would increase the validity of the WKY rat as a model of both stress-related GI disorders and co-morbid depression where there is a strong immune component.

The objective of this study was to assess the differential impact of an acute immune challenge on behaviour and neurochemical systems known to be consistently influenced by infection in the genetically stress-prone WKY rat compared to the Sprague Dawley strain commonly used as control [16,38,39]. Here we note that a genetically stressful background interferes with the "normal" response to LPS giving us an insight into the aberrant immune response in stress-related disorders.

2. Methods

2.1. Animals

Male Wistar Kyoto (WKY) and Sprague Dawley (SD) rats (Harlan, UK) (250–300 g) were used in these studies. These animals were group housed, five per cage. The animals were allowed to habituate to the facility in University College Cork for one week before experiments commenced. They were housed in plastic cages (15 × 22 × 9 cm). The animal room remained temperature controlled (20 ± 1 °C) and 12 h light/dark cycle (lights on at 7 a.m.). All experiments were approved by the Animal Experimentation Ethics Committee (AEEC) of University College Cork and carried out in accordance with the Cruelty to Animals Act 1876 and European Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes.

2.2. Experimental design

All studies were carried out in the morning between 9 a.m. until 12 p.m.

2.2.1. Visceral pain study

Four groups (n = 10 per group) were used to evaluate if a stress-sensitive background made the animals more susceptible to the effects of LPS (0.1 mg/kg i.p.) on visceral pain ((1) WKY + saline, (2) SD + saline, (3) WKY + LPS, (4) WKY + LPS). Two hours after LPS was administered colorectal distension (CRD) was performed. This dose and time-point were chosen because it is sufficient time to cause an elevation in the pro-inflammatory cytokines IL-1β and TNF-α [40], which are major mediators in visceral pain [35].

2.2.2. Sickness behaviours study

A different cohort of animals were used to assess sickness behaviours. Again four groups (n = 10 per group, groups as above) were used to assess the effect of strain difference on LPS (0.1 mg/kg i.p.). Basic behaviours analysed were social interaction, food and water consumption as well as temperature at two, six, and 24 h following treatment.

2.2.3. Neurochemical, corticosterone and cytokine analysis study

Another cohort of animals (experimentally naïve) (n = 8 per group) was used to collect samples in order to assess the central neurochemistry, peripheral cytokines and the stress hormone corticosterone at 0, 2, 6 and 24 h following treatment (LPS (0.1 mg/kg) or saline). Eight groups were used: (1) WKY + saline (0 h), (2) SD + saline (0 h), (3) WKY + LPS (2 h), (4) SD + LPS (2 h), (5) SD + LPS (6 h), (6) SD + LPS (6 h), (7) WKY + LPS (24 h), (8) SD + LPS (24 h). This strategy of using saline treated groups at one time-point for sample analysis has been used elsewhere [41].

2.3. Colorectal distension

This was carried out as previously described [42,43]. As before the two parameters measured were: (i) the threshold pressure (mmHg) that evokes visually identifiable visceral pain behaviour, and (ii) the total number of visceral pain behaviours. Postures defined as visceral pain-related behaviours were stretching, abdominal retractions and/or abdominal withdrawal reflex. The animals were tested in a random fashion and the experimenter was blind to the individual groups.

2.4. Sickness behaviour study

Sickness behaviour is a set of changes that occur during an infection [27]. Of these behaviours the commonly measured ones include food and water consumption [44] and social interaction [45]. These behaviours are consistently decreased following administration of LPS making them reliable measures of sickness behaviour and response to infection [27].

Rats were handled five days before the experiment, to minimise stress reactions to manipulations, and individually isolated 24 h before the first behavioural test. They were also acclimatised to the test conditions to reduce reactions to novelty. Each animal received the appropriate treatment and 2, 6 and 24 h afterwards behaviour was analysed: Social interaction, temperature, food and water intake were analysed as previously described [46].

2.4.1. Social interaction

Juvenile rats (Fisher) (21–28 days) which were group housed were used to assess social interaction of the test rats. These rats were introduced into the home cage of the rat being tested for 4 min. Different juveniles were introduced to the same rat over the different sessions. The amount of time spent by the test rat following, grooming, or sniffing the juvenile was recorded. Social exploration was defined as the total time spent performing all of these behaviours. Rectal temperature was also taken before each behavioural session.

2.5. Determination of neurotransmitter concentration in the dorsal raphe nucleus

The dorsal raphe nucleus (DRN) was excised from the brain by taking a slice of the fresh frozen brain that contained the midbrain and a micro-punch was used to dissect out the DRN. Neurotransmitter concentrations were determined using a modification of a previously described procedure [47]. Briefly, the DRN was sonicated in 500 μl of chilled mobile phase spiked with 4 ng/40 μl of N-Methyl 5-HT (Sigma Chemical Co., UK) as internal standard. The mobile phase contained 0.1 M citric acid, 0.1 M sodium dihydrogen phosphate, 0.01 mM EDTA (Alkem/Reagecon, Cork), 5.6 mM octane-1-sulphonic acid (Sigma) and 9% (v/v) methanol (Alkem/Reagecon), and was adjusted to pH 2.8 using 4 N sodium hydroxide (Alkem/Reagecon). Homogenates were then centrifuged at 14,000 rpm for 15 min at 4 °C and 40 μl of the supernatant injected onto the HPLC system which consisted of a SCL 10-Avp system controller, LC-10AS pump, SIL-10A autoinjector (with sample cooler maintained at 4 °C), CTO-10A oven, LECD 6A electrochemical detector (Shimadzu) and an online Gastorr Degasser (ISS, UK). A reverse-phase column (Kinetex 2.6u C18 100 × 4.6 mm, Phenomenex) maintained at 30 °C was employed in the separation (Flow rate 0.9 ml/min), the glassy carbon working electrode combined with an Ag/AgCL reference electrode (Shimdazu) was operated a +0.8 V and the chromatograms generated were analysed using Class-VP 5 software (Shimadzu). The neurotransmitters were identified by their characteristic retention times as determined by standard injections which were run at regular intervals during the sample analysis. Analyte: Internal standard peak height rations were measured and compared with standard injections and results were expressed at ng of neurotransmitter per g fresh weight of tissue.

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