General and Comparative Endocrinology 204 (2014) 203–210

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

High feather corticosterone indicates better coccidian infection resistance in greenfinches

Elin Sild [1], Richard Meitern, Marju Männiste, Ulvi Karu, Peeter Hõrak *

Department of Zoology, Institute of Ecology and Earth Sciences, Tartu University, Vanemuise 46, 51014 Tartu, Estonia

ARTICLE INFO

Article history:
Received 10 March 2014
Revised 21 April 2014
Accepted 19 May 2014
Available online 2 June 2014

Keywords:
Captivity tolerance
Coccidiosis resistance
Experimental infection
Feather corticosterone
Personality
Stress

ABSTRACT

Differential exposure or sensitivity to stressors can have substantial effects on the variation in immune responsiveness of animals. However, the questions about the causes and consequences of these processes have remained largely unclear, particularly as regards wild animals and their natural pathogens. Here we ask how a potential marker of stress responses, the feather corticosterone (CORT) content, reflects the resistance to an experimental infection with natural coccidian parasites in wild-caught captive greenfinches (*Carduelis chloris*). CORT content of tail feathers grown in captivity correlated positively with a behavioural measure of captivity-intolerance, i.e., the amount of damage accrued to tail feathers in captivity that results from flapping against cage bars. This finding is consistent with an idea that feather CORT reflects the amount of stress experienced during feather growth. Experimental infection with heterologous coccidian strains increased feather CORT levels. Birds with highest feather CORT levels appeared most resistant to new infection, assessed on the basis of parasite oocyst shedding at the peak phase of infection. Birds with highest feather CORT levels also cleared the infection faster than the birds with lower feather CORT levels. These findings provide the first evidence about positive covariation between feather CORT and resistance to a natural pathogen in a wild bird species. Assuming that feather CORT levels reflect circulating hormone titres, these findings suggest that parasite-mediated selection may contribute to maintenance of phenotypes with high corticosterone responsiveness to stress, despite potential negative behavioural consequences.

1. Introduction

In the face of continual attacks by parasites and pathogens, some individuals manage to cope with their infections better than others. Understanding the causes and consequences of this variation is among the most pertinent issues in evolutionary ecology and medicine. Immune function of vertebrates is tightly integrated with stress responses, stress being defined as a physiological and behavioural state engaged to endure, avoid, or recover from an aversive stimulus or condition (Martin, 2009). Stress is an intrinsic part of life for all organisms, and dealing successfully with stressors is what enables survival (Dhabhar, 2002). Responses to stressors involve activation of the hypothalamus–pituitary–adrenal (HPA) axis, which leads to increased synthesis and release of glucocorticoid hormones (Sapolsky et al., 2000).

The question of why stress responses are so tightly related to immunity is intriguing. Current adaptive explanations clarify why certain immune responses are enhanced in response to certain acute stressors, such as encounters with predators or aggressive conspecifics. In such a situation the benefits of preparing the immune system for invasion of pathogenic microbes entering via wounds is obvious (Dhabhar and McEwen, 1997). Similar immunoenhancement is followed to exposure to pathogenic cues (e.g., Schaller et al., 2010). However, the questions about why other types of stressors and chronic stress in general appear immunosuppressive lack definite answers (Martin, 2009; Segerstrom, 2007; Miller et al., 2002). For instance, assuming that the CORT response serves to "turn off" heightened immune responses that could result in autoimmune damage if sustained over long periods (Sapolsky et al., 2000; Martin, 2009) still leaves the issue about whether the benefits of avoidance of immunopathology would normally outweigh the costs of infection in immunocompromised organisms under natural conditions (e.g., Graham et al., 2005). Energy savings would be a viable explanation for stress-induced immune suppression only if savings could be gained rapidly. However, down-regulation of the immune system cannot be rapid, as the system is a diffuse network of cells and tissues (Martin, 2009).

A further layer of complexity in interrelationships between stress and immune responses involves coping styles or animal

* Corresponding author. Fax: +372 7375830.
E-mail address: horak@ut.ee (P. Hõrak).
[1] Present address: Molecular Ecology & Evolution Lab, Department of Biology, Lund University, Ecology Building, Sölvegatan 37, SE-223 62 Lund, Sweden.

http://dx.doi.org/10.1016/j.ygcen.2014.05.026
0016-6480/

personalities (Koolhaas, 2008; Segerstrom, 2007). Birds with proactive personalities have relatively bold and fast behavioural responses and relatively low corticosterone stress responses to stimuli, whilst birds with reactive personalities have relatively passive, shy and slow behavioural responses and large corticosterone responses (Cockrem, 2007). Proactive coping styles tend to be coupled with efforts to prevent or manipulate stressors, high sympathetic nervous system activity, and a Th1 biased (inflammatory) immune system. Reactive coping styles entail passive acceptance of stressors, high stress reactivity, and a Th2 biased (humoral) immune responses (Koolhaas, 2008; Korte et al., 2005; Martin, 2009) (but see Martins et al., 2007). There is thus a reason to expect that individual differences in disease resistance relate to behavioural phenotypes.

Understanding the nexus between stress, behaviour, and immune responses is particularly hampered by three major obstacles. First, the majority of the research on stress and immunity is performed on highly domesticated and often inbred strains of laboratory animals where (perhaps unintended) selection for tameness can efficiently eradicate behavioural phenotypes (Koolhaas et al., 2010). Despite several merits of such model systems, the ecological and evolutionary relevance of the major findings obtained in artificial populations with reduced variation of behaviour, stress responsiveness and immunity remains open (Martin, 2009). Second, a standard approach in immunological and immunoecological research involves assessment of immune function on the basis of organismal responses to artificial, non-replicating pathogens. This enables researchers to study the consequences of immune responses separately from the effects of parasites *per se*. The implicit assumption of such an approach, namely that immunoresponsiveness correlates with the ability to resist disease, or at least indicates the relative "strength" of the immune system, however, has been challenged (Saks et al., 2006; Owen and Clayton, 2007; Adamo, 2004).

Third, assessment of stress levels or responses on the basis of glucocorticoid hormone concentrations in blood appears a complicated task due to the rapid nature of the stress response. The major drawback with measuring plasma CORT is its rapid rise (in minutes) immediately following capture or handling of an animal (Romero and Reed, 2005). This complicates the interpretation of the individual differences in stress hormone levels. Clearly, assessment of plasma CORT is valuable due in part to the ability to measure stress-induced changes in CORT as well as basal or long-term CORT under standardized conditions, but this may not always be an advantage when comparing CORT titres against some more stable measure of immune function. Measuring stress hormone levels from feathers may help to overcome these problems, since feather CORT is expected to reflect plasma hormone levels during the period of feather growth, and thus to integrate variation in baseline level, magnitude of the stimulated response, time course of the stress response and the number of stressors experienced (Bortolotti et al., 2008; Lattin et al., 2011).

In the current study we investigate the connections between stress and immunity in a model system that avoids all three major obstacles outlined above. We ask whether experimental infection with a natural pathogen (coccidian intestinal parasites) affects CORT levels in the feathers of wild-caught captive greenfinches and how the feather CORT levels of experimental birds relate to resistance to infection. Coccidians are directly transmitted protozoan parasites that inhabit the epithelium of the intestine and directly inhibit the uptake of essential dietary components. They belong to the most prevalent of avian parasites (Greiner, 2008), causing massive production loss in poultry industry (Chapman, 2014). A host becomes infected when it ingests oocysts that have been passed in the faeces of another host. The oocyst excysts in the epithelial cells of intestinal mucosa and liberates sporozoites from its contents. The sporozoites penetrate the cells of the host's small intestine and reproduce asexually. In case of passerine birds, the first-generation sporozoites may also invade liver, spleen and lungs (Giacomo et al., 1997). In the epithelial cells of intestine, each generation of asexual reproduction produces multiple merozoites that infect new cells. This stage of the infection can result in destruction of massive numbers of cells in the host's small intestine and, ultimately, lead to the host's death (e.g., Sironi, 1994). In wild birds, coccidian infestation can affect both the physical appearance, via reduction in the signalling value of morphological traits, and the behaviour of an individual (reviewed by Pap et al., 2011).

The first line of defence against coccidian infection is represented by phagocytes that produce cytokines and consequently promote the development of cell-mediated immunity. Cytokines such as IL-12, IFN-γ, IL-1β and TNF-α are associated with inflammatory Th1 responses. IL-10 that favours the development of Th2 responses and thus plays an important role in preventing the development of strong responses driven by Th1-type cytokines and consequently reduces immune-mediated damage, is also involved (reviewed by Alcala-Canto et al., in press). Coccidiosis is a suitable model for immunoecological research because reproduction of parasites can be stopped with coccidiostatic drugs, which enables standardizing the infection status of hosts and later reinfection with parasite strains isolated from different donor individuals (e.g., Hõrak et al., 2006).

We focus on CORT content of tail feathers grown during the experiment. Feather CORT is sensitive to experimental manipulations of hormone levels (Lattin et al., 2011; Hõrak et al., 2013; Fairhurst et al., 2013) and thus expectedly reflects the experience of events that elevate circulating CORT levels during feather growth. To study the link with behaviour, we assessed the extent of damage to tail feathers accrued in captivity. Extent of tail damage was suggested to reflect captivity-intolerance in greenfinches and this trait was shown to correlate with a frequency of flapping bouts against cage walls (Sild et al., 2011). The same study also showed that greenfinches that inflicted greater extent of damage to their tail feathers exhibited compromised immune responsiveness in terms of reduced capability for producing antibody response to a novel *Brucella abortus* antigen and reduced oxidative burst capability of phagocytes, which suggests a possibility of CORT-induced immune suppression in birds with most damaged tail feathers. We thus predicted that the extent of tail damage accrued in captivity correlates positively with feather CORT content as an indicator of susceptibility to stress. We had no directional predictions about how feather CORT would relate to resistance to experimental coccidian infection, not least because the impact of CORT on immunity can differ between immune branches (e.g., Stier et al., 2009). Although our previous study has suggested that increased sensitivity to captivity stress is associated with suppression of the components of inducible innate immunity and Th1-type antibody responsiveness (Sild et al., 2011), it is not known whether similar pathways of immune activation also confer resistance to coccidiosis. Previous studies of experimental coccidiosis in passerine birds have shown that immune responsiveness towards artificial antigens is not necessarily a good predictor of infection resistance (Saks et al., 2006; Pap et al., 2009). Design of the current experiment provided us an opportunity to study the link between stress and an ecologically relevant pathogen.

2. Methods

2.1. Study protocol and infection

Fifty-six male wild greenfinches were captured in mist-nets at bird feeders in a garden in the city of Tartu (58° 22′ N; 26° 43′ E) on 28 and 29 December 2010 (days -6 and -5 with respect to