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Locally elevated cortisol in lymphoid organs of the developing zebra finch but not Japanese quail or chicken

Matthew D. Taves [a, b, *], Jennifer A. Losie [a], Titissa Rahim [a], Kim L. Schmidt [e, f], Benjamin A. Sandkam [g], Chunqi Ma [a], Frederick G. Silversides [d], Kiran K. Soma [a, b, c]

[a] Dept. of Psychology, University of British Columbia, Vancouver, BC, Canada
[b] Dept. of Zoology, University of British Columbia, Vancouver, BC, Canada
[c] Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, BC, Canada
[d] Agassiz Research Centre, Agriculture and Agri-Food Canada, Agassiz, BC, Canada
[e] Dept. of Biology, University of Western Ontario, London, ON, Canada
[f] Advanced Facility for Avian Research, University of Western Ontario, London, ON, Canada
[g] Department of Biological Sciences, Simon Fraser University, Vancouver, BC, Canada

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ABSTRACT

Glucocorticoids are important for production of functional lymphocytes and immunity. In altricial neonates, adrenal glands are unresponsive and local glucocorticoid synthesis in lymphoid organs may be necessary to support lymphocyte development. Precocial neonates, in contrast, have fully responsive adrenal glucocorticoid production, and lymphoid glucocorticoid synthesis may not be necessary. Here, we found that in altricial zebra finch hatchlings, lymphoid organs had dramatically elevated endogenous glucocorticoid (and precursor) levels compared to levels in circulating blood. Furthermore, while avian adrenals produce corticosterone, finch lymphoid organs had much higher levels of cortisol, an unexpected glucocorticoid in birds. In contrast, precocial Japanese quail and chicken offspring did not have locally elevated lymphoid glucocorticoid levels, nor did their lymphoid organs contain high proportions of cortisol. These results show that lymphoid glucocorticoids differ in identity, concentration, and possibly source, in hatchlings of three different bird species. Locally-regulated glucocorticoids might have species-specific roles in immune development.

1. Introduction

Animals exhibit dramatic variation along a spectrum of developmental strategies, ranging from undeveloped altricial offspring that are completely dependent on their parents, to developed and independent precocial offspring (Starck and Ricklefs, 1998). Avian and mammalian offspring rely heavily on innate immunity and maternal antibodies for protective immunity in early life, because lymphocytes that provide antigen-specific immunity are not yet mature and functional (birds, Davison et al., 2011; Klasing and Leshchinsky, 1999; mammals, Levy, 2007; Pilorz et al., 2005). A large proportion of lymphocytes are produced during early life (Adkins et al., 2004; Davison et al., 2011), creating a critical period during which environmental conditions can have long-term programming effects on adaptive immunity (Hodgson and Coe, 2006). The mechanisms underlying this developmental programming, however, are largely unknown.

Glucocorticoids (GCs) are steroid hormones (Fig. 1) that mediate environmental effects on immunity (Martin, 2009) and are critical for the production of functional T lymphocytes that are sufficiently responsive to foreign antigens (Mittelstadt et al., 2012). Glucocorticoids antagonize signaling of the T cell antigen receptor (Vacchio et al., 1994; Jamieson and Yamamoto, 2000; Van Laethem et al., 2001), and thus promote the survival of developing lymphocytes with higher affinity for self peptide in the context of major histocompatibility complex (MHC) molecules (Ashwell et al., 2000). Circulating GCs in the blood may not be sufficient for development

Abbreviations: GC, glucocorticoid; GR, glucocorticoid receptor; HAW, Hawaiian strain of Japanese quail; HPLC, high-performance liquid chromatography; MR, mineralocorticoid receptor; P0, posthatch day 0; PCR, polymerase chain reaction; RIA, radioimmunoassay; SEM, standard error of the mean; SHRP, stress hyporesponsive period; SPE, solid-phase extraction.

* Corresponding author. Dept. of Zoology, #4200-6270 University Blvd, Vancouver, BC, V6T 1Z4, Canada.
E-mail address: taves@zoology.ubc.ca (M.D. Taves).

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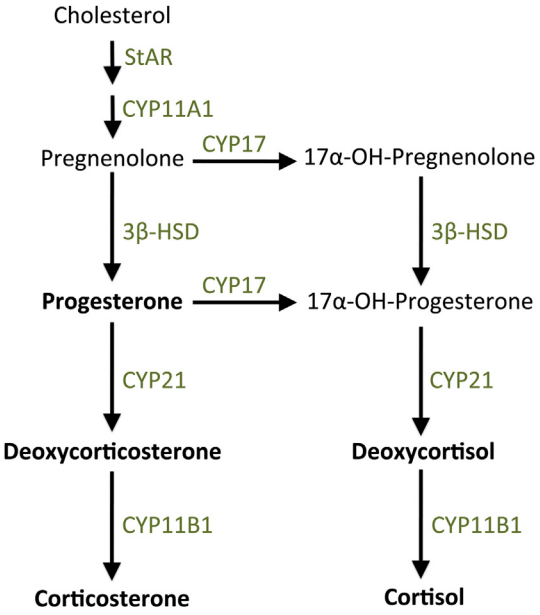

Fig. 1. Simplified glucocorticoid-synthetic pathway. Steroids quantified in this study are shown in bold, and steroidogenic enzymes are shaded in green. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of competent lymphocytes, as mouse pups and songbird nestlings (like altricial neonates of other species) experience a stress hyporesponsive period (SHRP) during which circulating GCs are extremely low and unresponsive (Schmidt et al., 2003; Wada, 2008). However, murine lymphoid organs synthesize GCs (Lechner et al., 2000; Pazirandeh et al., 1999; Vacchio et al., 1994), resulting in locally elevated GC levels in these organs (Taves et al., 2015). Interestingly, murine lymphoid organs can have high levels of cortisol, as well as the predominant circulating murine GC, corticosterone (Taves et al., 2015). High cortisol levels are also seen in lymphoid organs (thymus, bursa of Fabricius, and spleen) of an altricial bird, the zebra finch (Schmidt and Soma, 2008), where the predominant circulating avian GC is also corticosterone. Together, these findings suggest potentially distinct roles of cortisol and corticosterone in developing lymphoid organs.

Precocial offspring, in contrast, have responsive circulating GCs (Brown and Spencer, 2013; Starck and Ricklefs, 1998), which may be sufficient to support normal lymphocyte production. Circulating GCs of precocial offspring, like circulating GCs of adults, are highly responsive to environmental conditions. Chronically increased or decreased GC levels in the blood could alter lymphocyte development, resulting in lymphocytes with corresponding increased or decreased reactivity (here, we use "reactivity" to refer to the overall strength of antigen receptor signaling of the lymphocyte repertoire, and the resulting likelihood of recognizing and responding to a given antigen). In this way, changes in circulating GCs might be a mechanism by which environmental conditions program adaptive immunity. Alternatively, lymphoid organs of developing chickens appear to synthesize cortisol *in vitro* (Lechner et al., 2001), indicating that chicken offspring may also require locally-elevated GC levels for development of functional lymphocytes.

Here, we aimed to determine whether lymphoid GCs of three different avian species follow similar patterns of local elevation (relative to circulating GCs) during post-hatch immune development, or whether lymphoid GC levels instead follow distinct, species-specific patterns. We measured two endogenous GCs: corticosterone, the predominant circulating avian GC, and cortisol, a GC classically thought to be absent in birds, but which we have found in avian lymphoid organs (Schmidt and Soma, 2008). Locally elevated lymphoid GC levels (in lymphoid organs relative to circulating blood) across species would suggest that the maintenance of stable GC concentrations is critical for lymphocyte development. However, if local GC levels remain similar to circulating GC levels, this would suggest that environmental conditions play a stronger role in determining the GC levels to which lymphoid organs are exposed. Furthermore, we also quantified GC precursors, 11-deoxycorticosterone (deoxycorticosterone), 11-deoxycortisol (deoxycortisol), and progesterone. The presence of locally elevated GCs together with their precursors suggests that local synthesis from upstream precursors could play a major role in local GC elevation (Taves et al., 2011a, 2015). In addition, these steroids can independently bind to and regulate activity of steroid receptors (Gomez-Sanchez, 2014). Samples were collected from the Zebra finch *(Taeniopygia guttata)*, the Japanese quail *(Coturnix coturnix japonica)*, and the chicken *(Gallus gallus domesticus)*. Comparative studies of developmental strategies have largely focused on birds, providing substantial background information and thereby making them well suited to explore the role of steroids in immune development. Furthermore, the developmental effects of glucocorticoids have been extensively investigated in these three domestic species, making them especially useful models (Schoech et al., 2011).

We examined birds as hatchlings and as juveniles, as lymphocyte production is greatest in early life (Orkin and Zon, 2008) and lymphoid organs regress with age (Davison et al., 2011; Glick, 1956). For both quail and chicken we included two strains, to look for differences both within species and across species. Because lymphocyte selection is extremely stringent to achieve immunocompetence while avoiding autoimmunity, we hypothesized that offspring of all three bird species would have locally elevated GCs and GC precursors in developing lymphoid organs relative to levels in circulating blood.

2. Materials and methods

2.1. Subjects

Zebra finches are born naked, immobile, with closed eyes, and with highly immature tissues, and parents provide food, warmth, and protection against predators and parasites (Starck and Ricklefs, 1998; Zann and Bamford, 1996). Altricial development (perhaps in part due to the stress hyporesponsive period) facilitates rapid posthatch growth (Ricklefs, 1979; Wada, 2008; Wada et al., 2009). Due to immature sensory systems (Herrmann and Bischof, 1988), immature physiological responses to stimuli (Wada et al., 2009) and buffering of environmental conditions by parents (Lindström, 1999), altricial hatchlings have limited interactions with environments beyond the nest. Parents even engage in behaviors to protect the nest from parasites (Petit et al., 2002).

Zebra finches in this study were from a captive colony maintained at the Advanced Facility for Avian Research at the University of Western Ontario. Finches were housed on a 14 h: 10 h light:dark cycle, and had ad libitum access to grit, cuttlefish bone, water, and seed (11% protein, 6% lipid; Living World premium finch seed). Breeding pairs were given a nest box, and received daily supplements of hardboiled eggs, cornmeal, and bread. Hatchling finches were collected from nest boxes on the day of hatch (P0, or posthatch day 0), and juveniles (still housed with parents) were collected at P30, which is approximately one-third of the age of sexual maturity (gonads mature at approximately P90).

Japanese quail and chickens are born feathered, mobile, with open eyes, and able to actively forage for themselves (McNabb and McNabb, 1977; Nichelmann and Tzschentke, 2002; Ottinger, 2001).