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Lymphoid organs of neonatal and adult mice preferentially produce active glucocorticoids from metabolites, not precursors

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ABSTRACT

Glucocorticoids (GCs) are circulating adrenal steroid hormones that coordinate physiology, especially the counter-regulatory response to stressors. While systemic GCs are often considered immunosuppressive, GCs in the thymus play a critical role in antigen-specific immunity by ensuring the selection of competent T cells. Elevated thymus-specific GC levels are thought to occur by local synthesis, but the mechanism of such tissue-specific GC production remains unknown. Here, we found metyrapone-blockable GC production in neonatal and adult bone marrow, spleen, and thymus of C57BL/6 mice. This production was primarily via regeneration of adrenal metabolites, rather than *de novo* synthesis from cholesterol, as we found high levels of gene expression and activity of the GC-regenerating enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), but not the GC-synthetic enzyme CYP11B1. Furthermore, incubation with physiological concentrations of GC metabolites (11-dehydrocorticosterone, prednisone) induced 11β-HSD1- and GC receptor-dependent apoptosis (caspase activation) in both T and B cells, showing the functional relevance of local GC regeneration in lymphocyte GC signaling. Local GC production in bone marrow and spleen raises the possibility that GCs play a key role in B cell selection similar to their role in T cell selection. Our results also indicate that local GC production may amplify changes in adrenal GC signaling, rather than buffering against such changes, in the immune system.

1. Introduction

Glucocorticoids are steroid hormones synthesized by the adrenal glands that circulate through the blood to coordinate organismal physiology. Circulating glucocorticoids are especially responsive to psychological and physiological stressors, and have pleiotropic effects on neural, metabolic, and immune function (Sapolsky et al., 2000). Acute, transient elevation of circulating glucocorticoids has positive effects on neural and immune function, enhancing cognition and memory (Lupien et al., 2009) and mobilizing immune cells to better respond to pathogens (Bowers et al., 2008), enhancing the ability to survive adverse conditions. In contrast, chronic elevation of circulating glucocorticoids has harmful effects on cognition and mental health (Lupien et al., 2009), suppresses immune responses to pathogens (Dhabhar, 2009), and induces lymphocyte apoptosis (Ashwell et al., 2000). This lymphocyte susceptibility to glucocorticoid-induced apoptosis is clearly demonstrated by the notable reduction in thymus and spleen masses in response to sustained stressors (Selye, 1936; Jellinck et al., 1997).

While circulating, adrenal-derived glucocorticoids act on tissues throughout the body, some tissues can locally produce their own glucocorticoids, allowing tissue-specific regulation of glucocorticoid concentrations and signaling (Taves et al., 2011a). Such local glucocorticoid production is best studied in the thymus, where glucocorticoids antagonize signaling through the T cell antigen receptor (TCR) (Iwata et al., 1991; Vacchio et al., 1994; Van Laethem et al., 2001) and are critical for the selection of immunocompetent T cells (Lu et al., 2000; Mittelstadt et al., 2012). Interestingly, the thymus

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expresses all of the upstream enzymes required for *de novo* conversion of cholesterol into active glucocorticoids (Vacchio et al., 1994; Pazirandeh et al., 1999; Fig. 1). Enzyme activity has also been shown in the thymus, via metyrapone-blockable glucocorticoid production (Vacchio et al., 1994; Lechner et al., 2000; Fig. 1). As metyrapone is commonly used as an inhibitor of the glucocorticoid-synthetic enzyme CYP11B1 (P450c11B1), these data together have led to the conclusion that the thymus produces glucocorticoids from upstream precursors (hereafter, "synthesis"), and that this activity decreases with age (Taves et al., 2011a).

However, more recent work has shown that the thymus also expresses 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), an enzyme that converts inactive glucocorticoid metabolites [11-dehydrocorticosterone (DHC), and cortisone] into active glucocorticoids (Nuotio-Antar et al., 2006; Qiao et al., 2008; Fig. 1). Like upstream CYP11B1, 11β-HSD1 and 11β-HSD2 are also inhibited by metyrapone (Sampath-Kumar et al., 1997; Hostettler et al., 2012). These data raise the possibility that metyrapone-blockable glucocorticoid production might also involve thymus production of glucocorticoids from metabolites (hereafter, "regeneration"), rather than synthesis from precursors.

Glucocorticoid synthesis is independent of circulating steroids (Schmidt et al., 2008), while glucocorticoid regeneration is instead dependent on circulating adrenal-derived glucocorticoid metabolites (Chapman et al., 2013) that can change acutely in response to stressors (Obut et al., 2009) or chronically in psychological disorders (Weber et al., 2000). Thus, the relative contributions of CYP11B1 and 11β-HSD1 could determine how strongly environmental conditions and stressors influence T cell selection. Furthermore, we have previously measured endogenous glucocorticoid levels in developing and adult mice, and have found that in addition to the thymus, glucocorticoids are also locally elevated in the developing bone marrow and spleen (Taves et al., 2015). This indicates that local glucocorticoid production occurs *in vivo*, and that in addition to being critical in T cell development, it may also be important in the development and differentiation of other hematopoietic cells, such as B cells (Gruver-Yates et al., 2013), myeloid cells (Schaer et al., 2002; Trottier et al., 2008), and erythrocytes (Bauer et al., 1999).

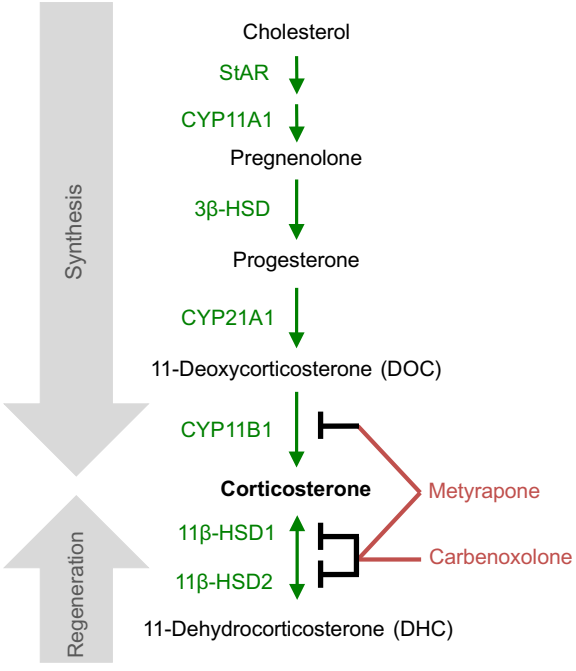

Fig. 1. Simplified glucocorticoid-metabolic pathway. Steroid names are in black, steroidogenic enzyme names and their activities are shaded in green, and the enzyme inhibitors (metyrapone and carbenoxolone) are shaded in red. Here, we define precursor (DOC) conversion to corticosterone as "synthesis," and metabolite (DHC) conversion to corticosterone as "regeneration." The combined effects of synthesis and regeneration are referred to as "production". (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Here, we investigated whether murine bone marrow, spleen, and thymus locally express glucocorticoid-metabolic enzymes, and whether this changes with age. We also examined whether glucocorticoid production ("production" referring to both synthesis and regeneration) occurs via synthesis from upstream precursors, or via regeneration of metabolites. As glucocorticoid precursors are elevated in neonatal lymphoid organs (Taves et al., 2015), we hypothesized that neonatal lymphoid organs would produce glucocorticoids via synthesis, and that adult lymphoid organs would have decreased synthesis and increased regeneration. Finally, as a functional test of glucocorticoid production, we quantified glucocorticoid receptor-mediated lymphocyte apoptosis in the presence of physiologically relevant substrate concentrations.

2. Materials and methods

2.1. Study 1: Endogenous gene expression of glucocorticoid-metabolic enzymes

2.1.1. Subjects

Female and male C57BL/6 mice were bred and housed at the Centre for Disease Modeling, a specific pathogen-free facility at the University of British Columbia. Mice were group housed with corncob bedding, under a 14:10 light:dark cycle, with *ad libitum* water and food (Teklad, diet 2918 for adults, diet 2919 for breeders). All protocols were approved by the UBC Animal Care Committee (A12-0119). All tissue samples were collected in the morning (between 0800 and 1200 h), to reduce possible diurnal variation in steroid levels.

2.1.2. Tissue collection

Neonates (5 days old, with day 0 being the morning that pups were first present) or adults (2–3 months old) were deeply anesthetized with isoflurane in oxygen (<2 min) and euthanized by rapid decapitation. Decapitation and trunk blood collection was completed in less than 3 min after initial disturbance. Immediately after euthanasia, spleen and thymus were collected, cleaned of fat and connective tissue, and bone marrow was flushed from femurs with ice-cold PBS. Tissues were then snap-frozen on dry ice and stored at −80 °C.

2.1.3. Endogenous glucocorticoid concentrations

This work was performed using the same mouse line as in our previous work (Taves et al., 2015), but our previous animal facility (Wesbrook Animal Unit) was closed and the mouse colony was rederived in the Centre for Disease Modeling (detailed in Taves (2015)). Therefore, we conducted a pilot study, to test whether neonates in this facility exhibited similar local elevation of lymphoid glucocorticoid levels, as previously reported (Taves et al., 2015). We again found that progesterone and corticosterone concentrations were locally elevated in neonatal lymphoid organs, compared with concentrations in circulating blood. However, unlike our previous work, we did not detect high concentrations of cortisol. Thus, we focused this series of experiments on corticosterone, which exhibited highly similar patterns of local elevation in lymphoid organs of neonates from both animal facilities.