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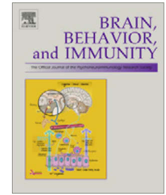

Corticosterone and cortisol binding sites in plasma, immune organs and brain of developing zebra finches: Intracellular and membrane-associated receptors

Kim L. Schmidt [a,b,*], Jessica L. Malisch [d], Creagh W. Breuner [d,1], Kiran K. Soma [a,b,c,1]

[a] Department of Psychology, University of British Columbia, Vancouver, BC, Canada
[b] Graduate Program in Neuroscience, University of British Columbia, Vancouver, BC, Canada
[c] Department of Zoology, University of British Columbia, Vancouver, BC, Canada
[d] Division of Biological Sciences, Organismal Biology and Ecology, University of Montana, Missoula, MT, USA

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ABSTRACT

Glucocorticoids (GCs) affect the development of both the immune and nervous systems. To do so, GCs bind to intracellular receptors, mineralocorticoid receptors (MR) and glucocorticoid receptors (GR). In addition, GCs bind to membrane-associated corticosteroid receptors (mCR). Two well-known GCs are corticosterone and cortisol. Whereas corticosterone is the primary GC in zebra finch plasma, cortisol is the primary GC in zebra finch lymphoid organs and is also present in the brain and plasma during development. Here, we characterized binding sites for corticosterone and cortisol in plasma, liver, lymphoid organs, and brain of developing zebra finches. In tissues, we examined both intracellular and membrane-associated binding sites. For intracellular receptors, there were MR-like sites and GR-like sites, which differentially bound corticosterone and cortisol in a tissue-specific manner. For mCR, we found little evidence for membrane-associated receptors in immune organs, but this could be due to the small size of immune organs. Interestingly, cortisol, but not corticosterone, showed a low amount of specific binding to bursa of Fabricius membranes. For neural membranes, corticosterone bound to one site with low affinity but a relatively high B_{max}, and in contrast, cortisol bound to one site with high affinity but a lower B_{max}. Our results indicate that intracellular and membrane-associated receptors differentially bind corticosterone and cortisol suggesting that corticosterone and cortisol might have different roles in immune and nervous system development.

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1. Introduction

Glucocorticoids (GCs) have important effects on the development of many physiological systems, particularly the immune and nervous systems. For example, in the immune system, developmental GC treatment can suppress cell-mediated immune responses (Loiseau et al., 2008; Rubolini et al., 2005), and GCs may regulate positive and negative selection of $CD4^+$ $CD8^+$ thymocytes (Ashwell et al., 2000; Vacchio et al., 1999). In the brain, neonatal GC treatment can alter corticosteroid receptor levels in the brain of rats, affecting the hypothalamic–pituitary–adrenal (HPA) axis in adulthood (Welberg et al., 2001; Liu et al., 2001). Lastly, neonatal GC administration and stressors can cause long-term impairments in learning and cognition in a variety of species (Spencer and Verhulst, 2007; Aisa et al., 2007).

GCs affect the development of the immune system and brain by binding to receptors in target tissues. There are two intracellular receptors that bind GCs: the Type 1 or mineralocorticoid receptor (MR), which binds endogenous GCs with high affinity, and the Type 2 or glucocorticoid receptor (GR), which binds endogenous GCs with lower affinity (Funder, 1992; de Kloet et al., 1990). The intracellular corticosteroid receptors are part of the steroid receptor superfamily and act as ligand-dependent transcription factors (Carson-Jurica et al., 1990). GR is expressed in cells of the immune system and brain during development. In the immune system, GR

* Corresponding author. Address: Department of Biology, University of Western Ontario, 1151 Richmond St. N, London, ON, Canada N6A 5B7. Fax: +1 519 850 2923.
E-mail address: kschmi5@uwo.ca (K.L. Schmidt).
[1] Co-principal investigators.

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is present in lymphocytes of embryonic mice and regulates thymocyte selection (Ashwell et al., 2000; Jondal et al., 2004). For example, in transgenic mice, thymocyte GR over-expression decreases thymocyte number (Pazirandeh et al., 2005). In addition, administering the mammalian GR antagonist RU 486 to cultured thymic cells increases apoptosis of immature thymocytes (Vacchio et al., 1994). In the brain, expression of GR and MR in the hippocampus of rats and mice increases across development and reaches adult-like levels between the second and third weeks (Pryce, 2008). Intracellular corticosteroid receptors are involved in neuronal migration (Fukumoto et al., 2009) and synapse maturation (Kumamaru et al., 2008) during development.

In addition to the cytosolic receptors, GCs can bind to membrane-associated receptors (Song and Buttgereit, 2006; Trueba et al., 1987). In the immune system, membrane-associated corticosteroid receptors (mCR) have been detected in human peripheral blood mononuclear cells (Tryc et al., 2006), T cells (Löwenberg et al., 2008), and murine lymphoma cells (Gametchu, 1987; Gametchu et al., 1999) and may mediate GC-induced apoptosis (Stahn and Buttgereit, 2008). In the brain, mCR have been well characterized in adult rough-skinned newts (*Taricha granulosa*; Orchinik et al., 1991) and tiger salamanders (*Ambystoma tigrinum*; Orchinik et al., 2000). In newts, GCs bind to mCR in medullary neurons to rapidly inhibit reproductive behavior (Rose et al., 1993; Moore and Orchinik, 1994). Brain mCR have also been examined in adult house sparrows (*Passer domesticus*; Breuner and Orchinik, 2001, 2009) and rats (Moutsatsou et al., 2001; Towle and Sze, 1983). Few studies have examined mCR in the immune system or brain during development (Sze and Towle, 1993).

The two well-known GCs that bind to corticosteroid receptors are corticosterone and cortisol. Corticosterone is considered the primary circulating GC in some species (e.g., rats, mice, birds), whereas cortisol is considered the primary circulating GC in other species (e.g., humans, fish). The prevailing belief is that a species has just one predominant GC, corticosterone or cortisol, and that these two GCs are interchangeable, in the sense that they act via similar mechanisms and have similar effects. However, there are species that have both hormones present in equal concentrations, for example, New Zealand white rabbits (Szeto et al., 2004). In addition, the ratio of these two GCs can vary depending on the site of sampling. In humans, the corticosterone to cortisol ratio is five to sixfold higher in cerebrospinal fluid (Raubenheimer et al., 2006) and post-mortem brain tissue (Karssen et al., 2001) than in plasma, which suggests that corticosterone might have more important effects on the human brain than previously thought (Raubenheimer et al., 2006). Furthermore, although corticosterone is the predominant circulating GC in adult birds (Wingfield et al., 1982; Nakamura and Tanabe, 1973), cortisol is the predominant GC in lymphoid organs of developing zebra finches (*Taeniopygia guttata*) and is also present in the plasma and brain (Schmidt and Soma, 2008; Schmidt et al., 2009). These findings suggest that the identity of the predominant GC can be organ-specific and age-specific (Schmidt and Soma, 2008) and raise the hypothesis that corticosterone and cortisol may exert different effects (Schmidt et al., 2008) by differentially binding to corticosteroid receptors. For example, in young chickens, the GR in the bursa of Fabricius (hereafter bursa; the primary avian immune organ that produces B cells; Glick and Olah, 1982; Cooper et al., 1966) have a higher affinity for cortisol than corticosterone (Sullivan and Wira, 1979).

There were two goals of the current study. First, we wanted to determine whether there are both intracellular and membrane-associated corticosteroid receptors in immune organs and brain during development. Second, we wanted to determine if corticosteroid receptors differentially bind corticosterone and cortisol. Cortisol is the more abundant GC in zebra finch lymphoid organs during development, so we predicted that there would be a receptor in lymphoid organs that preferentially binds cortisol. Also, locally-synthesized steroids are more likely to act via membrane-associated receptors (Schmidt et al., 2008). For example, in mice, 17β-estradiol is the principal estrogen in the blood, but 17α-estradiol may be more abundant in the brain and binds with higher affinity to a membrane-associated receptor (Toran-Allerand et al., 2005). Therefore, we predicted that cortisol may be the preferred ligand for a mCR in immune organs.

2. Methods

2.1. Subjects

Research was carried out under a University of British Columbia (UBC) permit, following the guidelines of the Canadian Council on Animal Care. Subjects were male and female juvenile zebra finches ($n = 110$ total). The zebra finch is an altricial songbird species that is commonly used for studies of avian endocrinology, neurobiology, and stress physiology (Goodson et al., 2005). Zebra finches serve as an excellent species for this study because plasma GC levels have been well characterized in adulthood (Wada et al., 2008) and development (Schmidt and Soma, 2008; Wada et al., 2009). Further, developmental changes in both corticosterone and cortisol levels in immune organs and brain have been determined (Schmidt and Soma, 2008; Schmidt et al., 2009).

Zebra finches were sampled at approximately 30 days post-hatch (P30) (mean ± SEM = 30.21 ± 0.18 d). Cortisol levels in immune tissues are elevated, relative to corticosterone levels, at P30 (Schmidt and Soma, 2008). Moreover, this is the youngest age at which we could collect a sufficient amount of tissue for the binding assays. Breeding pairs of adult zebra finches were given millet seeds, water, grit, and cuttlefish bone *ad libitum*. Breeding pairs were also given a daily supplement consisting of boiled chicken eggs, cornmeal, and bread. The light cycle was 14L:10D (lights on at 08:00). Temperature was held at ∼23 °C and relative humidity at ∼50%. At P30, zebra finches had fledged the nest but remained in the cage with their parents. Zebra finches reach nutritional independence at ∼P30 and reproductive maturity at ∼P90 (Zann, 1996).

2.2. Chemicals

[1,2,6,7-^{3}H]Corticosterone (specific activity = 70 Ci/mmol, NET399001MC) and [1,2,6,7-^{3}H]cortisol (specific activity = 72.4 Ci/mmol, NET396001MC) were purchased from Perkin-Elmer Life Sciences (Waltham, MA, USA). Unlabeled steroids were purchased from Steraloids (Newport, RI, USA). Mitotane, RU 486, activated charcoal, Trisma base, HEPES, sucrose, EDTA, polyethylenimine, molybdic acid, glycerol, bovine serum albumin, and dithiothreitol were all purchased from Sigma–Aldrich (Oakville, ON, Canada).

2.3. Sample collection and preparation

2.3.1. Plasma

All subjects were sampled between 08:00 and 12:00. Plasma was collected to characterize the corticosteroid-binding globulin (CBG). Whole blood was collected by puncturing the brachial vein with a 26-gauge needle and collecting blood into heparinized micro-hematocrit tubes. All blood samples were collected within 3 min of opening the cage door. Blood was centrifuged at 10,000 rpm for 10 min. Plasma was collected and stored at −20 °C. For equilibrium saturation binding and competition assays, plasma from multiple subjects was pooled. Prior to the binding assays, plasma was charcoal stripped (1% Norit A charcoal and 0.1% dextran in 50 mM Tris buffer) to remove endogenous steroids.

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