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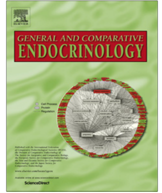

Beeswax corticosterone implants produce long-term elevation of plasma corticosterone and influence condition

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ABSTRACT

Glucocorticoids can play a critical role in modulating life-history trade-offs. However, studying the effects of glucocorticoids on life-history often requires experimentally elevating plasma glucocorticoid concentrations for several weeks within normal physiological limits and without repeated handling of the animal. Recently, implants made of beeswax and testosterone (T) were shown to have release dynamics superior to some currently available T implants, and these beeswax implants dissolved, eliminating the need to recapture the animal. We evaluated the utility of beeswax implants containing four different dosages of corticosterone (CORT; the primary glucocorticoid in birds) and their effect on several condition indices in a captive colony of zebra finches (*Taeniopygia guttata*). The three implants with the greatest CORT doses (0.05, 0.1, and 0.5 mg) produced spikes in plasma CORT concentrations 20 h after treatment, but were within the limits that zebra finches may normally experience. The 0.5 mg CORT implant elevated plasma CORT between typical baseline and restraint stress levels reported in other studies of zebra finches for the entire 35 day experiment. Birds in the 0.5 mg implant group were heavier, had greater furcular fat scores, and had lower hematocrit than birds in the control and other CORT implant groups. Beeswax CORT implants are a low cost method of elevating plasma CORT for a prolonged time. Furthermore, because there is no need to remove these implants at the end of a study, this method may be amenable to studies of free-ranging animals.

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1. Introduction

In vertebrates, glucocorticoids are pivotal in regulating daily and seasonal responses to changing energetic demands and in mediating the response to unpredictable events (Landys et al., 2006; Wingfield et al., 1998). Circulating levels of glucocorticoids can change rapidly in response to variations in the physical or social environment, and lead to a suite of physiological and behavioral changes that can influence fitness (Crossin et al., 2016). Recent work in birds has indicated that variation in plasma corticosterone (CORT; the main glucocorticoid in birds) concentrations can influence parental care (Crossin et al., 2012), offspring sex ratios (Bonier et al., 2007), offspring development (Butler et al., 2010; Müller et al., 2009b), immune function (Butler et al., 2010; Råberg et al., 1998) and individual fitness (reviewed in Bonier et al., 2009). Thus, there is keen interest in determining the causal role of this hormone in regulating a range of life-history trade-offs (Crossin et al., 2016). Such experimental manipulations require a means to manipulate plasma CORT in the field and laboratory for prolonged durations.

The growing interest in CORT has led to an increasing number of studies experimentally manipulating plasma CORT concentrations in the field and laboratory, but such manipulations are frequently problematic (Crossin et al., 2016; Fusani, 2008). Ideally, a manipulation would elevate plasma CORT consistently within the desired range (between baseline and stress-induced levels) over a specific time scale and be minimally invasive. Injections elevate CORT in the short-term, but require repeated handling and capture of birds, which is logistically difficult and the repeated handling and injections could also affect the hypothalamic-pituitary-adrenal axis and therefore plasma CORT concentrations (Loiseau et al., 2008; Müller et al., 2009a). The passive administration of CORT through food or water is non-invasive but requires that animals are held in captivity, and the CORT dose will vary among individuals depending on the amount of water and food consumed (Breuner et al., 1998; Müller et al., 2009a). Oral treatments also produce a high, but relatively brief (≈1 h), spike in plasma CORT (Spencer and Verhulst, 2007). Silastic implants generally elevate

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plasma CORT to acute stress levels for 1–3 days (but see Ouyang et al., 2013) before levels return to baseline (Newman et al., 2010; Shahbazi et al., 2014; Wingfield and Silverin, 1986). Osmotic pumps are relatively large and elevate plasma CORT for over a week, but they have the downside of producing a spike in plasma CORT 24–48 h following implantation (Horton et al., 2007). Osmotic pumps and silastic implants will remain in the animal long-term if it is not recaptured, which could pose ethical issues. Commercially available CORT pellets (hereafter 'pellets') are advantageous because they are biodegradable and, thus, no part remains in the animal long-term, but they are relatively costly compared to silastic implants (Fusani, 2008; Müller et al., 2009a). Pellets also have the benefit of producing a spike in plasma CORT approximately half that produced by a silastic implant and can elevate plasma CORT concentrations from a few days to several weeks (Bonier et al., 2007; Fusani, 2008; Müller et al., 2009b).

Recently, Quispe and colleagues (2015) developed implants made of beeswax to administer testosterone (T). Compared to silastic implants and pellets, beeswax implants produced a lower spike in plasma T 24 h following implantation and more consistently elevated plasma T for 2 weeks following implantation (Quispe et al., 2015). Beeswax implants provide the additional advantage of dissolving inside the animal (Quispe et al., 2015). We evaluated the use of beeswax implants for long-term elevation of plasma CORT using a captive population of zebra finches (*Taeniopygia guttata*). We prepared implants containing four different CORT dosages as well as blank implants and evaluated plasma CORT levels and several condition indices to determine how CORT dosages affected condition. Based on the results of Quispe et al. (2015), we expected that plasma CORT would remain elevated above baseline for 2 weeks following implantation, with an initial spike in plasma CORT within the range produced by acute stress. Because CORT supports gluconeogenesis primarily through the breakdown of lipids and proteins (Sapolsky et al., 2000), we predicted that individuals receiving implants containing more CORT would decrease fat and muscle scores, and lose body mass compared to controls. We further predicted that hematocrit would increase in individuals receiving more concentrated CORT implants because elevated plasma glucocorticoids increase erythropoiesis in poultry and mice (Olanrewaju et al., 2007, 2006; Voorhees et al., 2013).

2. Methods

2.1. Animals, housing conditions, and experimental design

We kept adult zebra finches in temperature-controlled rooms (25 ± 1 °C) on a 14:10 h light: dark cycle with food (mixed seeds; Kaytee, Chilton, WI, USA) and water *ad libitum*. We used 24 male and 24 female zebra finches and randomly assigned each bird to one of six treatment groups while equally distributing the sexes among treatments ($n = 8$ per group): 0.01 mg CORT implant, 0.05 mg CORT implant, 0.1 mg CORT implant, 0.5 mg CORT implant, 0 mg CORT implant (implant control), and no implant (total control). We selected these dosages based on the ability of commercially available implants of similar concentrations to moderately increase plasma CORT in other passerines (Bonier et al., 2007; Pravosudov, 2003). We divided the birds between 8 cages, with each cage including one bird from each treatment group and a variable sex ratio. Cages were distributed among three separate rooms.

Five to 7 days after assigning the birds to cages and allowing them to acclimate to their social group, we collected a pre-treatment blood sample from each bird to quantify baseline plasma CORT. We aimed to bleed birds at each time point within 3 min of entering the room (Romero and Reed, 2005) and staggered bleeds over three consecutive days to achieve this. On a sample day, all of the birds in one cage from each of the three rooms were bled on average 2:13 ± 0.05 min (range 0:53–5:36 min) after entering the room. We collected blood samples between 8:30 AM and 9:45 AM by puncturing the brachial vein with a 26½ gauge needle and collecting ⩽150 μL of blood from each bird in heparinized capillary tubes. Within an hour of collection, we centrifuged samples at 10,000 rpm for 5 min and stored the separated plasma at −80 °C until hormone assay (see below).

Fourteen days after collecting the pre-treatment samples, we inserted one implant into each bird (total control birds received no implant or incision, but were handled similarly to the other birds). To insert implants subcutaneously on the flank, we first swabbed the surgical site with povidone-iodine and topically applied benzocaine anesthetic, then made an incision in the skin. After inserting the implant, we closed the incision using cyanoacrylate adhesive (surgi-loc 2oc, Meridian Animal Health, Omaha, NE, USA) and returned the bird to its cage. We performed all implant surgeries between 1 PM and 3 PM, staggering the birds over three days. The day of implantation was designated as day 0 and we collected the first blood samples the morning following implantation (day 1; an average of 19.5 h after implantation), and subsequent samples on days 7, 20, and 35 post-implantation as described previously.

2.2. Implant preparation

We made the implants following a modification of the protocol described by Quispe et al. (2015). We autoclaved beeswax (90% by weight; Sigma-Aldrich, St. Louis, MO, USA, cat. # 243221) and hardened peanut oil (10% by weight; Sigma-Aldrich, St. Louis, MO, USA, cat. # 93967) in glass vials and mixed them in a water bath at 67 °C. Once the beeswax/peanut oil mixture was melted, we added crystalline CORT (cat. # 27840, Sigma-Aldrich, St. Louis, MO, USA) dissolved in ethanol, and allowed the solution to mix until the ethanol evaporated. We then poured the beeswax/peanut oil solution into a 3 mL syringe with a Luer-lok tip (Becton Dickinson, Franklin Lakes, NJ, USA). Once the mixture had cooled and partially solidified in the syringe, we extruded it through the tip to produce a 1.75 mm diameter cylinder, which we cut into 10 mm lengths. To create implants with different doses of CORT, we made separate batches of the beeswax/peanut oil mixture and to each we added ethanol with sufficient CORT (calculated according to the average mass of a 10 mm long beeswax cylinder and the amount of CORT dissolved in the ethanol) such that a 10 mm length contained 0.01 mg, 0.05 mg, 0.1 mg, or 0.5 mg of CORT. Control implants were made with ethanol that contained no CORT.

2.3. Post-implantation care

We experienced 8% mortality (4 of 48 birds) and two birds had their implant removed prior to the completion of the study due to issues with the incision site. One bird from each group that received an implant containing CORT died but no birds from the implant control group died. These deaths may be due to some combination of infection following implantation, the stress of the surgery, and the effects of elevated CORT. As a result of these deaths, we treated all of the birds with the antibiotic Baytril (at 200 mg/L in water) beginning one week following implantation and had no further mortality. An additional six birds displayed some sickness behavior or issues with their surgical site that led us to individually dose them with Baytril (15 mg/kg). Deaths following implantation have occurred in other studies (Shahbazi et al., 2014), and we encourage future implant studies to report mortality so that

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