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Research paper

Physiological and behavioral effects of exogenous corticosterone in a free-ranging ectotherm

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ABSTRACT

In the face of global change, free-ranging organisms are expected to experience more unpredictable stressors. An understanding of how organisms with different life history strategies will respond to such changes is an integral part of biodiversity conservation. Corticosterone (CORT) levels are often used as metrics to assess the population health of wild vertebrates, despite the fact that the stress response and its effects on organismal function are highly variable. Our understanding of the stress response is primarily derived from studies on endotherms, leading to some contention on the effects of chronic stress across and within taxa. We assessed the behavioral and hormonal responses to experimentally elevated stress hormone levels in a free-ranging, arid-adapted ectotherm, the Southern Pacific rattlesnake (*Crotalus helleri*). Plasma CORT was significantly elevated in CORT-implanted snakes 15 days after implantation. Implantation with CORT did not affect testosterone (T) levels or defensive behavior. Interestingly, we observed increased defensive behavior in snakes with more stable daily body temperatures and in snakes with higher plasma T during handling (tubing). Regardless of treatment group, those individuals with lower baseline CORT levels and higher body temperatures tended to exhibit greater increases in CORT levels following a standardized stressor. These results suggest that CORT may not mediate physiological and behavioral trait expression in arid-adapted ectotherms such as rattlesnakes.

1. Introduction

Stress is defined broadly as a state an organism experiences when coping with unpredictable or adverse conditions (Wingfield, 2005). To cope with such conditions, organisms typically respond by elevating levels of circulating glucocorticoids such as cortisol and/or corticosterone (CORT; Sapolsky et al., 2000). Responses to stress can be further split into (A) acute, short term responses, and (B) chronic, sustained responses, with variation in the effects based on the magnitude of the response (Dickens and Romero, 2013). Acute responses are generally mounted during brief encounters with predators or aggressive conspecifics (Sapolsky et al., 2000), while chronic responses are often due to prolonged unfavorable environmental and physiological conditions (Busch and Hayward, 2009; Wingfield et al., 1998). Chronic stress can influence the expression of a suite of traits in many taxa, including but not limited to reproduction, immune function, behavior, thermoregulation, and protein metabolism (reviewed in Wingfield et al., 1998; Martin, 2009; Wingfield and Sapolsky, 2003; Landys et al., 2006). Significant changes in any of these traits could affect an individual's survival and reproductive success (Escribano-Avila et al., 2013; Jessop et al., 2013a).

Life history theory posits that trade-offs exist between survival and reproduction, and that organisms prioritize success in one area at the expense of the other (Stearns, 1989). Pervasive in the literature is the idea that CORT mediates an emergency life history stage by prioritizing immediate survival and suppressing reproductive measures, leading to a broader assumption that acute CORT responses are adaptive and thus increase lifetime reproductive success, while prolonged elevation of CORT above baseline (i.e. a chronic response) has negative effects on both survival and reproduction (Bonier et al., 2009; Sapolsky et al., 2000; Wingfield et al., 1998). Physiological stress responses and baseline CORT levels are

Abbreviations: CORT, corticosterone; T_b, body temperature; T, testosterone.

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thus often used to assess the health of free-ranging populations of animals and to predict future growth or reproduction (Busch and Hayward, 2009; Jessop et al., 2013b). A major caveat to using CORT as a metric for assessing population health is that the effects of high levels of CORT are not consistent across taxa (Cockrem, 2013), and their effects on organismal health are often assumed rather than measured. Knowledge of the effects of CORT on organismal survival, specifically in the context of defensive behavior against potential predators, is limited (Herr et al., 2017; Spencer et al., 2015; Thaker et al., 2010). There is an obvious need to evaluate the direct effects of CORT on traits influencing various aspects of fitness (Breuner et al., 2008), especially in the context of chronic CORT elevation, where sustained high CORT levels may have damaging effects (Baker et al., 2013). In order for CORT measures to be useful across scientific disciplines, it is also important to measure the behavioral and physiological effects of CORT across taxa (Dantzer et al., 2014).

The majority of studies assessing effects of elevated CORT have focused on the acute stress responses and effects of CORT in endothermic vertebrates (Baker et al., 2013; Busch and Hayward, 2009; Jessop et al., 2013b). Fewer studies have focused on how CORT levels respond to and mediate chronic stress, and fewer still have investigated this in terrestrial ectotherms (DeNardo and Licht, 1993; DeNardo and Sinervo, 1994a,b; Juneau et al., 2015). Due to their relatively slow metabolism, ectotherms may endure chronic CORT elevation for longer periods of time than endotherms (Landys et al., 2006), and this may result in different responses or effects between the two groups. Conditions that induce chronic stress in wild vertebrates (e.g., habitat loss or environmental toxins; Baker et al., 2013) are expected to increase over the coming decades (Wingfield, 2008). Thus, it is important to understand how different taxa respond to chronic stress and especially whether elevated CORT levels convey an appropriate measure of organismal health.

Key to our ability to test hypotheses about effects of chronic stress responses is the ability to experimentally manipulate CORT levels. In many studies, this involves exposing organisms to repeated acute stressors to simulate a chronic stress response (reviewed in Dickens and Romero, 2013). However, relative to these types of techniques, sustained CORT via direct experimental manipulation may more accurately mimic CORT levels during chronic stress. Experimental studies of this nature on free-ranging organisms, especially ectotherms, are rare, partially due to difficulties in experimentally inducing chronically elevated CORT levels (Sopinka et al., 2015).

In this study, we evaluated the use of CORT implants to induce prolonged elevation of CORT and investigated the behavioral and hormonal effects of elevated CORT in a free-ranging, arid-adapted ectotherm. We chose the Southern Pacific rattlesnake (*Crotalus helleri)* as our study organism because it lives in dense populations and is resilient to radio-telemetry and monitoring, allowing for repeated sampling of wild individuals with minimal observer-induced impact (Holding et al., 2014a). Ecologically, it is important to understand whether CORT plays a major role in mediating fitness-related traits in organisms known to survive periods of chronic stress, such as rattlesnakes and other arid-adapted ectotherms which can survive drought and low food availability (McCue, 2007). To investigate the effects of CORT on rattlesnake physiology and behavior, we implanted radio-telemetered male *C. helleri* with CORT-filled or blank implants and subsequently measured circulating CORT, testosterone (T), and defensive behaviors for 30 days post-implantation. If CORT plays a role in mediating traits related to survival and reproduction, then rattlesnakes implanted with CORT should have decreased T (Moore and Jessop, 2003; Jones and Bell, 2004; Wingfield and Sapolsky, 2003), increased defensive behavior (Herr et al., 2017), and an increase in both baseline and stress-induced CORT levels (Dupoué et al., 2013; Sykes and Klukowski, 2009).

2. Methods

Thirty adult male *C. helleri* were captured through visual encounter surveys at the University of California at Santa Barbara's Sedgwick Reserve in Santa Ynez, Santa Barbara County, California (34.6928°N, 120.0406°W, elevation: 290 m) from mid-April to early May 2015. At this reserve, artificial fresh water sources are available for wildlife year-round, thus snakes in this study were unlikely to be chronically stressed due to reduced food or water availability (Capehart et al., 2016; Jessop et al., 2013b; Sperry and Weatherhead, 2008). The site is primarily cattle-grazed valley oak savannah habitat, with areas of chaparral and coastal sagebrush. Rattlesnakes were collected under California Department of Fish and Wildlife Scientific Collecting Permit # SC-13134, and experimental procedures were approved by the California Polytechnic State University Institutional Animal Care and Use Committee (Protocol #1416) and the University of California at Santa Barbara Institutional Animal Care and Use Committee (Protocol #Taylor 1415, Animal Activity #027).

After capture, snakes were transported to California Polytechnic State University for processing and housed individually in 30″ × 12″ × 12″ Visionarium cages (Vision Products) with heat pads, hide boxes, and water *ad libitum*. Snakes received intramuscular passive integrated transponder tags (MUSICC Chip, AVID Identification Systems, Inc., Norco, CA, USA), and the three basal rattle segments were filled with non-toxic acrylic paint to a predetermined color code for future identification. Snakes were anesthetized via isofluorane inhalation (Vet One, MWI, USA) and received intra-coelomic implants of a 3.25 g temperature data logger (Thermochron iButton model DS1922L#F50, Maxim Integrated, San Jose, California) and a radio-transmitter weighing 5.3, 11, or 13.5 g (Holohil Systems Ltd., Carp, Ontario, Canada) depending on snake body size. Combined weights of implants totaled less than 5% of snake total body mass. Snakes were released at the site of capture 1–2 days after surgery.

2.1. Experimental administration of exogenous corticosterone

Snakes were allowed to recover in the field for at least two weeks post-surgery prior to experimental manipulation in late May 2015. Snakes were divided into two mass classes at 800 g, which was the average and median weight for all snakes captured. Snakes greater than 800 g were given two 15 mm (large) implants (see below), and snakes less than 800 g received two 7.5 mm (small) implants. Snakes within each weight class were randomly assigned to either a treatment group or control group. Treatment snakes received implants filled with CORT, while control snakes received blank implants. Seven large snakes and eight small snakes were randomly assigned to the CORT treatment group and eight large and seven small snakes were assigned to the control group.

Implants were made by plugging one end of a 15 mm or 7.5 mm silastic diffusion tubing (Dow Corning, Clarkesville, TN, USA: 1.47 mm inner, 1.96 mm outer diameter) with 2 mm of silicone caulking (Momentive Performance Materials Inc., Huntersville, NC), then allowed to dry for 24 h. The tubing was then filled with crystalline CORT (Sigma C2505-500 mg Lot# SLBJ5337V), plugged with 2 mm of silicone on the open end, and allowed to dry for 24 h. Control implants were left empty and plugged with silicone at both ends. 15 mm treatment implants averaged 6.1 mg CORT, while 7.5 mm treatment implants averaged 3.6 mg CORT.

Implants were soaked in 0.9% saline solution for 12 h prior to implantation to facilitate CORT release. Each snake received two