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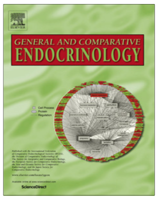

Sources of variation in plasma corticosterone and dehydroepiandrosterone in the male northern cardinal (*Cardinalis cardinalis*): II. Effects of urbanization, food supplementation and social stress

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ABSTRACT

Perturbations in an organism's environment can induce significant shifts in hormone secretory patterns. In this context, the glucocorticoid (GC) steroids secreted by the adrenal cortex have received much attention from ecologists and behaviorists due to their role in the vertebrate stress response. Adrenal GCs, such as corticosterone (CORT), are highly responsive to instability in environmental and social conditions. However, little is understood about how adrenal dehydroepiandrosterone (DHEA) is influenced by changing conditions. We conducted field experiments to determine how circulating CORT and DHEA vary during restraint stress in the male northern cardinals (*Cardinalis cardinalis*). Specifically, we examined how four different changes in the physical (urbanization and food availability) and social (territorial conflict, distress of a mate) environment affect CORT and DHEA levels. The majority of cardinals responded to restraint stress by increasing and decreasing CORT and DHEA, respectively, however this depended on sampling context. Cardinals sampled from urban habitats had both lower initial and restraint stress CORT concentrations, but a comparable DHEA pattern to those sampled from a forest. Supplementing food to territorial males did not alter circulating initial DHEA or CORT concentrations nor did it change the response to restraint stress when compared to unsupplemented controls. Exposing cardinals to varying durations of song playback, which mimics a territorial intrusion, did not affect CORT levels, but did attenuate the DHEA response to restraint stress. Examining a larger dataset of males captured before, after or at the same time as their female mate, allowed us to address how the stress of a captured mate affected the male's CORT and DHEA response. Males showed elevated initial and restraint CORT and DHEA when their female mate was captured first. Taken together, these data demonstrate that both CORT and DHEA secretion patterns depends on environmental, and particularly current social conditions.

1. Introduction

Environmental instability has major consequences for the survival and reproductive success of organisms and thus being capable of coordinating an appropriate response to such stress is a necessity. In vertebrates, the hypothalamic–pituitary–adrenal (HPA) axis is an endocrine cascade activated by a stressor that regulates physiology and behavior that ultimately promote survival (Wingfield and Sapolsky, 2003). During HPA activation, the adrenal gland responds to pituitary-derived adrenocorticotropic hormone (ACTH) by secreting glucocorticoids (GCs), such as corticosterone (CORT) in birds (Sapolsky et al., 2000). These GCs regulate the reallocation of energy reserves towards vital physiological functions, promotes survival behaviors and suppresses immune functions and reproduction in a holistic effort to cope with environmental challenges (McEwen and Wingfield, 2003). The causes of environmental instability, though highly variable, can be broadly divided into two types: 1) those involving sudden changes in the physical environment that result in shifts in the resources in an area, such as food availability or changes in habitat structure; and 2) those involving intraspecific interactions that alter an individual's social standing, such as mating conflicts. Although, both forms of instability can alter GC secretion in wild animals, they differ in the degree to which they depend on context. For example, depletion of food resources is an energetic stressor that imposes direct and consistent physiological impacts on individuals (...) However,

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stress associated with a territorial challenge depends on the condition of the intruder and the resident, the nature of the interaction, quality of the territory, and the presence or absence of a female (. . .). Thus physical and social environments may represent different sources of variation in GC secretion.

The adrenal gland, in addition to GCs, also secretes the androgen precursor, dehydroepiandrosterone (DHEA) in response to ACTH (Boonstra et al., 2008; Kalimi et al., 1994; Soma et al., 2014a,b), although this may vary across species (Bélanger et al., 1990, 2002; van Weerden et al., 1992). Ascertaining the biological role of DHEA has been elusive, in part because a specific receptor has not been explicitly identified (Labrie et al., 2001; but see Webb et al. (2006) and Widstrom and Dillon (2004). Thus DHEA presumably is converted to active androgens (e.g., testosterone or T) and estrogens (e .g., estradiol or E_2) to exert its effects (Soma et al., 2014a,b). Research has also been limited due to the lack of an appropriate research model as mice and rats have essentially undetectable DHEA levels (van Weerden et al., 1992). In humans and red squirrels (*Tamiasciurus hudsonicus*), acute stress increased circulating DHEA levels (Boonstra et al., 2008; Strous et al., 2006), but no effects on DHEA concentrations were observed in the song sparrow (*Melospiza melodia*) (Newman et al., 2008; Soma and Wingfield, 2001). Functionally, rodent and *in vitro* studies have suggested DHEA counteracts GC effects on neurons (Kimonides et al., 1999; McNelis et al., 2013; Kalimi et al., 1994), and studies in song sparrows and Siberian hamsters (*Phodopus sungorus*) suggest DHEA may promote aggressive behaviors during non-breeding contexts (Soma et al., 2014a,b). Despite the growing body of literature, still little is understood concerning how instability in the physical and social environmental impacts DHEA concentrations.

To assess environmental instability, field studies of vertebrates often compare initial (i.e., baseline) steroid levels with those after a period of handling stress. This widely-used method has garnered important information concerning the ecological roles of steroids, and in particular GCs, in wild animals (Deviche et al., 2010; Fokidis et al., 2011b; French et al., 2008; Holding et al., 2014; Pereyra and Wingfield, 2003; Pravosudov et al., 2002; Refsnider et al., 2015; Woodley et al., 2014). In recent years, how animal endocrine systems respond to urbanization has garnered significant interest, and here the CORT response to stress is paramount. As urban landscapes encroach on natural environments they present numerous novel stressors to wildlife including: human disturbance (Fernández-Juricic et al., 2005); traffic (Bautista et al., 2004); noise (Slabbekoorn and Peet, 2003); feral animal predators (Baker et al., 2008; Woods et al., 2003); introduced and invasive competitors (Shochat et al., 2010); pollutants (Janssens et al., 2001); street lighting (Longcore and Rich, 2004); and warmer temperatures (McLean et al., 2005). Several bird species have been examined in detail regarding the effects of urbanization on HPA activity, however generalizations have been difficult to make due to variation between species (Bonier, 2012). In some species, urban populations suppress their stress-induced CORT secretion (Partecke et al., 2006), presumably to avoid the costs of overstimulation in a ‘‘stressful” environment. Other species show a greater CORT response to handling in urban areas (Fokidis et al., 2009; Schoech et al., 2007), perhaps to enable them to continually cope with stressors as they encounter them. Thus species-specific CORT responses may indicate whether birds are positively and negatively effected by the instability associated with urbanization. In contrast to CORT, no study has investigated variation in DHEA levels between cities and natural animal populations. Among the benefits cited for birds in urban areas is gaining access to higher and more predictable food resources which serves to decrease environmental instability (Fokidis et al., 2011a; Heiss et al., 2009). Food supplementation of free-living birds typically lowers baseline CORT levels and decrease the CORT response to handling stress (Clinchy et al., 2004; Schoech et al., 2007), whereas fasting imposes the opposite effects (Fokidis et al., 2013, 2012, 2011a). Fasting is also known to elicit an increase in plasma DHEA in both avian and mammalian species (Fokidis et al., 2013, Grasfeder et al., 2009), however the effects of food supplementation on DHEA in free-living animals have not been investigated.

Environmental instability associated with changing intraspecific social interactions also modulate patterns of GC secretion. Changes in circulating CORT levels during conspecific social challenges are usually tested using simulated territorial intrusion (STI) (Deviche et al., 2014, 2012; Newman and Soma, 2011). In many bird species, CORT levels remain constant during antagonistic interactions (Fokidis et al., 2011b), possibly since CORT may interfere with T secretion which may assist the individual in winning a territorial bout (Deviche et al., 2010). However, in species where T increases are not seen (e.g., those with short breeding seasons), CORT increases are observed instead because the bout is viewed as a stressor and/or because the response requires energy mobilization. Furthermore, some birds also exhibit increases in plasma DHEA levels during STI (Hau and Beebe, 2011; Hau et al., 2004). Another source of social instability is the distress of a mate (e.g., during a predatory event) often associated with distress calling vocalizations (Conover, 1994). Distress calls may serve as warnings to other birds (Conover, 1994; Hill, 1986), or may provide information about an individual’s quality to the predator (Laiolo et al., 2004). Observing one’s mate being captured may stimulate a stress response in the observer, particularly for species with strong pair bonds. However, the effect of pair distress on CORT and DHEA levels has received little, if any, attention from researchers, yet constitutes an important component of environmental instability.

The aim of this study was to examine how environmental instability in different contexts influences CORT and DHEA concentrations in free-living adult male northern cardinals (*Cardinalis cardinalis*) to determine the degree to which the responses of these adrenal steroids are parallel. Specifically, we compared plasma CORT and DHEA concentrations before and after restraint stress in four contexts of environmental instability: 1) between birds inhabiting urban and natural forest habitats; 2) between birds that were food supplemented with controls; 3) between birds challenged with varying durations of a STI; and 4) birds experiencing the distress of a captured mate with those that were not exposed to this stimulus. Northern cardinals are common resident songbirds in Central Florida, and both sexes actively defend territories year-round (DeVries et al., 2015, 2012, 2011; Jawor, 2007). Research on this species has focused on the regulation of year-round T secretion in both males and females, as both sexes behave similarly during territorial conflicts and both participate in parental duties thus suggesting a strong pair bond (DeVries and Jawor, 2013; DeVries et al., 2015, 2014, 2012, 2011; Jawor, 2007; Jawor et al., 2014; Jawor and MacDougall-Shackleton, 2008; Nealen and Breitwisch, 1997). In contrast to T, very little research has been done on other androgens in this species (but see Barron et al., 2012 and Owen et al., 2012). To our knowledge, no research has examined DHEA in cardinals. This study is the first to examine how the context of environmental instability influences the secretory patterns of both CORT and DHEA in a free-living species.

2. Materials and methods

2.1. Ethics statement

Each experiment was conducted under United States Geological Survey Bird Banding Laboratory permit #23847, Florida Fish, and