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Regulation of plasma testosterone, corticosterone, and metabolites in response to stress, reproductive stage, and social challenges in a desert male songbird

Pierre Deviche [a,*], Benjamin Beouche-Helias [b], Scott Davies [a], Sisi Gao [a], Samuel Lane [a], Shelley Valle [a]

[a] School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA
[b] Universite de Poitiers, Faculte des Sciences Fondamentales et Appliquees, Poitiers F-86022, France

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ABSTRACT

In many male vertebrates, the secretion of reproductive (gonadal androgens) and adrenocortical (glucocorticoids) hormones varies seasonally and in response to environmental stimuli, and these hormones exert numerous behavioral and metabolic effects. We performed two field studies on adult male Rufous-winged Sparrows, *Peucaea carpalis*, a Sonoran Desert rain-dependent sedentary species, to (a) determine seasonal changes in initial (baseline) and acute stress-induced plasma testosterone (T), corticosterone (CORT), and two metabolites (uric acid and glucose) and (b) compare the effects of two types of social challenge (song playback or simulated territorial intrusion consisting of song playback plus exposure to a live decoy bird) on plasma T, CORT, these metabolites, and territorial behavior. Initial plasma T was higher during the summer breeding period than during post-breeding molt. Acute stress resulting from capture and restraint for 30 min decreased plasma T in breeding condition birds but not in the fall, revealing that this decrease is seasonally regulated. Initial plasma CORT did not change seasonally, but plasma CORT increased in response to acute stress. This increase was likewise seasonally regulated, being relatively smaller during autumnal molt than in the summer. We found no evidence that acute stress levels of CORT are functionally related to stress-depressed plasma T and, therefore, that plasma T decreases during stress as a result of elevated plasma CORT. Thirty minutes of exposure to simulated territorial intrusion resulted in different behavior than 30 min of exposure to song playback, with increased time spent near the decoy and decreased number of overhead flights. Neither type of social challenge influenced plasma T, thus offering no support for the hypothesis that plasma T either responds to or mediates the behavioral effects of social challenge. Exposure to both social challenges elevated plasma CORT, but simulated territorial intrusion was more effective in this respect than song playback. Plasma uric acid and glucose decreased during acute stress, but only plasma uric acid decreased during social challenge. Thus, an elevation in plasma CORT was consistently associated with a decrease in plasma uric acid, but not with a change in glycemia. These results enhance our understanding of the short-term relationships between T, CORT, and avian territorial behavior. They provide novel information on the endocrine effects of acute stress, in particular on plasma T, in free-ranging birds, and are among the first in these birds to link these effects to metabolic changes.

1. Introduction

In response to adverse conditions (i.e., stressors), vertebrates often activate their hypothalamic–pituitary–adrenal axis, resulting in elevated plasma glucocorticoids, and enter the "emergency life history stage" (Wingfield et al., 1998). The transition to this life history stage involves physiological and behavioral changes that promote survival and it is, therefore, thought to be adaptive (Sapolsky et al., 2000; Wingfield and Kitaysky, 2002). These changes include energy mobilization, increased cardiovascular tone, and sharpened cognition. Transition to the emergency life history stage can also affect reproductive processes, in particular the secretion of reproductive hormones, and studies suggest a role for glucocorticoids such as cortisol and corticosterone (CORT) in this effect. The rapid (i.e., within minutes to hours) rise in plasma CORT in response to acute stress concurs with a rapid fall in plasma testosterone (T) in fish (Pickering et al., 1987), amphibians

* Corresponding author. Fax: +1 480 965 6899.
E-mail address: deviche@asu.edu (P. Deviche).

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(Woodley and Lacy, 2010), mammals (Dong et al., 2004), and birds (Lynn et al., 2010; Wingfield et al., 1982), including male Rufous-winged Sparrows, *Peucaea carpalis* (Deviche et al., 2012a), the species investigated in the present study. Mammalian studies reveal a direct action of glucocorticoids on testicular endocrine function, showing that glucocorticoids can inhibit plasma T production by acting on interstitial (Leydig) testicular cells through a non-genomic action (Dong et al., 2004; Hu et al., 2008; Martin and Tremblay 2008). Avian testes contain glucocorticoid receptors and, similar to mammals, elevated plasma CORT may, therefore, acutely decrease plasma T in birds through a testis-mediated mechanism (Kwok et al., 2007).

In most free-ranging male vertebrates, plasma T varies seasonally such that levels are highest when males are competing for mates at the beginning of the breeding season (Landys et al., 2010; Van Duyse et al., 2003; Wingfield et al., 1987). Initial (often referred to as baseline) and stress-induced plasma CORT can also vary seasonally, but whether this is the case is species-specific. In some vertebrates, initial plasma CORT is elevated during energetically demanding times such as the early breeding season (Breuner and Orchinik, 2001; John-Alder et al., 2009; Place and Kenagy, 2000; Romero, 2002) or migration (Romero et al., 1997). In other male vertebrates initial plasma CORT does, however, not change seasonally (Landys et al., 2010). Likewise, stress-induced plasma CORT fluctuates seasonally in some male vertebrates (Breuner and Orchinik, 2001; Klukowski, 2011; Romero, 2002; Romero et al., 1997) but not in others (Place and Kenagy, 2000). In free-ranging birds, the CORT response to acute stress is often smaller during than outside the molt period (Cyr et al., 2008; Remage-Healey and Romero 2000; Romero, 2002; Romero et al., 1998a).

Studies on species exhibiting seasonal variation in plasma T and in initial and stress-induced plasma CORT provide an opportunity to investigate functional relationships between these hormones. Here we addressed this question using free-ranging adult male Rufous-winged Sparrows sampled at different reproductive stages. This Sonoran Desert passerine breeds during the summer monsoon (Lowther et al., 1999). In males, plasma T is seasonally highest in July and August, when birds are in breeding condition, and lower in September, after birds complete their breeding cycle (Deviche et al., 2006, 2012a). No study on Rufous-winged Sparrows has determined seasonal changes in plasma CORT. The first main objective of the present study was to examine seasonal variation in initial and stress-induced plasma CORT and to relate this variation to changes in initial and stress-induced plasma T in the same birds. We predicted that if plasma CORT changes seasonally and the changes are functionally related to plasma T, an inverse relationship would exist between these hormones on a seasonal basis and in response to acute stress.

In addition to fluctuating during acute stress, plasma CORT and T in free-ranging territorial males can be socially modulated. This modulation is often investigated by presenting males with a social challenge consisting of exposure to conspecific song recordings (song playback, SPB) or to a conspecific decoy male and conspecific song recordings (simulated territorial intrusion, STI). Exposure to SPB simulates an auditory social challenge whereas exposure to STI simulates a combined visual and auditory social challenge. Exposure to SPB or STI often provokes an aggressive response and in some cases, also elicits endocrine changes (Goymann et al., 2007; Landys et al., 2007). Several studies found that exposure to STI increases plasma CORT (Gill et al., 2008; Landys et al., 2007, 2010; Van Duyse et al., 2004). This exposure also stimulates plasma T in some species (Wingfield, 1994), but either reduces or has no effect on plasma T in others (Apfelbeck and Goymann 2011; Hau and Beebe, 2011; Landys et al., 2007, 2010). Likewise, exposure to SPB either increases (Wikelski et al., 1999) or does not influence plasma T, as was the case in the Rufous-winged Sparrow (Deviche et al., 2006) and in the congeneric Cassin's Sparrow, *Peucaea cassinii* (Deviche et al., 2012b). Elevated plasma T in response to social challenges may function to increase the subsequent persistence of aggressive responses (Challenge Hypothesis, Wingfield et al., 1987, 1990) and species-specific differences in endocrine responses to social challenges are postulated to be related to life history characteristics such as varying mating systems and brood numbers per season (Goymann et al., 2007; Hirschenhauser et al., 2003; Landys et al., 2007). However, reaching firm conclusions on this subject has been complicated by methodological differences between studies. In particular, little research has been carried out to compare the endocrine changes that are induced by exposure to SPB *vs.* STI within the same study and in the same species. To address this issue, the second objective of the present study was to compare the effects of auditory (SPB) vs. auditory plus visual (STI) stimulation on the aggressive behavior and on plasma T and CORT of male Rufous-Winged Sparrows in breeding condition. This comparison also offered the opportunity to further investigate relationships between plasma T and CORT and, therefore, to compare this relationship to that observed in the above seasonal study.

The downstream metabolic effects of stress-related endocrine changes are critical to determining whether these changes improve the chances of survival and fitness, but these effects have not been well investigated in free-ranging birds. Glucose is a main substrate for energy production. In birds, CORT may, in some circumstances, regulate plasma glucose, but information on this subject is conflicting. Acute stress in captive European Starlings, *Sturnus vulgaris*, increased plasma glucose during the night but not during the day and this effect is not observed in molting birds (Remage-Healey and Romero, 2001, 2002). In this species, CORT administration to captive molting birds during the day elevated plasma glucose, but the significance of this finding is obscured by the fact that the treatment resulted in supra-physiological plasma CORT (Remage-Healey and Romero 2002). Other studies likewise found that acute stress either increases (Corbel et al., 2010; Davies et al., 2013) or does not influence glycemia (Fokidis et al., 2011a; House Sparrow, *Passer domesticus*: M. Romero, personal communication), and Corbel et al. (2010) obtained no evidence for an interaction between CORT and the metabolic response (including plasma glucose) to stress in penguins. Uric acid is a product of protein degradation (Costantini 2008) and a potent antioxidant that is absorbed into tissues and oxidized to protect against tissue damage (Stinefelt et al., 2005). Stressful stimuli increase plasma uric acid in some species (Cohen et al., 2007; Tsahar et al., 2006), but has the opposite effect in others (Cohen et al., 2007; Davies et al., 2013). If CORT in free-ranging birds plays a major proximate role in regulating metabolic responses to acute stress – including glycemia and plasma uric acid, we predicted changes in plasma CORT to be consistently related to changes in the circulating levels of these metabolites across experimental conditions. To test this prediction, we measured plasma glucose and uric acid in free-ranging males across different reproductive stages, in response to social challenge, and before and after acute stress.

2. Materials and methods

2.1. Study site, species, capture, and blood sample collection

We sampled adult male Rufous-winged Sparrows between June 21st and September 26th 2012 in the Santa Rita Experimental Range, Pima County, Arizona, USA (altitude: 979 m; latitude: 31°49′N; longitude: 110°55′W). This sedentary species is territorial and responds behaviorally to SPB year-round (Lowther et al., 1999; personal observations).