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Cortisol and corticosterone exhibit different seasonal variation and responses to acute stress and captivity in tuco-tucos (*Ctenomys talarum*)

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ABSTRACT

In this work we aimed to evaluate variations in plasma glucocorticoids (GCs, cortisol and corticosterone) levels throughout an annual cycle in free-living male tuco-tucos (*Ctenomys talarum*) and compare their responses to acute and chronic stressors (trapping, manipulation, immobilization, confinement in a novel environment, transference to captivity). In addition, we used leukocyte profiles to allow discrimination between basal and stress-induced seasonal changes in GC concentrations. Our results showed that cortisol and corticosterone are differently affected by environmental stimuli in *C. talarum*. Both hormones showed different patterns of variation in the field and responses to captivity. Moreover, only cortisol was responsive to acute stressors. Leukocyte profiles indicated that animals were unstressed in the field and therefore, that we were able to measure basal, stress-independent, fluctuations in GC levels. GC concentrations were low in comparison to values frequently reported for other mammals. Our results suggest differentiated physiological roles for cortisol and corticosterone in our study species and further emphazise the complexity of GC physiology in wild mammals.

1. Introduction

Glucocorticoids (GCs, cortisol and/or corticosterone, depending on the species) are important hormones that serve many adaptive functions in vertebrates. GCs are secreted by the adrenal gland and regulate the availability of energy by influencing glucogenesis, glucose use and protein metabolism [7]. In addition, adverse environmental conditions (i.e., stressors) are known to induce the hypothalamic–pituitary–adrenal (HPA) axis to secrete GCs above basal levels, which seems critical to maintain or restore homeostasis during or after the challenge [41]. However, chronically-elevated GCs have a variety of deleterious effects upon health [40] and can suppress the hypothalamic–pituitary–gonadal (HPG) axis, affecting reproduction [44]. It has been demonstrated in free-living animals that GC concentration can be negatively related with survival [33,37], though the relationship between GC levels and fitness is not always clear [6,12]. An additional important finding of recent field studies is that free-living animals can seasonally modulate GC secretion, leading to differences in both baseline and stress-induced GC concentrations, though in mammals the pattern of GC release varies from species to species [35].

One unresolved question related to HPA axis physiology is why some mammals have both cortisol and corticosterone in detectable amounts in plasma (e.g., chipmunks, ground squirrels [10,31,38]). Since Kenagy and Place [24] pointed that the physiological meaning of two different plasma GC hormones should be studied little was done in order to shed light on this matter. From the scarce available information, it is widely assumed that cortisol and corticosterone share their physiological roles and that their relative importance depends on their concentrations in plasma [9,31,38]. One initial approach to evaluate if cortisol and corticosterone indeed accomplish the same functions in a given species may be to compare their responses to experimental treatments (including both acute and chronic stressors) and their patterns of variation under natural conditions. While similarities would confirm the accepted view of shared physiological functions, differences would suggest that both hormones do not completely overlap in their roles and exhibit some differentiation in function.

Another issue that deserves further clarification is the physiological meaning of the seasonal fluctuations in baseline GC levels [35]. Though baseline samples have been collected in a number of studies (defined as blood samples taken within 3 min of capture, see Section 2), the fact that the story of animals prior to capture can't be usually known prevents from assessing if these variations represent changes in strictly *basal* GC levels or are influenced by naturally-occurring stressors operating before the captures. This problem may be overcome by coupling data on GC levels with the determination of leukocyte profiles (or other confident stress indicators), if one has characterized such profiles during stress and unstressed conditions in the study species (see [16]). Specially,

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the neutrophil: lymphocyte (N:L) ratio is rapidly increased by stress and can be directly related to stress hormone levels [16]. Thus, whilst parallel seasonal increases in N:L ratios and GC levels would indicate increased stress levels, variation in GC levels concurrently with relatively low and constant N:L ratios may indicate that strictly basal GC concentrations are being modulated on a seasonal basis .

The subterranean rodent *Ctenomys talarum* (talas tuco-tucos, see [23] and below *Species characteristics*) constitutes an interesting model to explore the ecophysiology of the HPA axis in mammals. Both cortisol and corticosterone are present in plasma of other hystricomorph rodents such as guinea pigs [15] and chinchillas [32], though in the colonial tuco-tuco *Ctenomys sociabilis* only corticosterone was present in significant amounts [46]. The species show high levels of intraspecific aggression [50] suggesting important roles for GCs in the mobilization of energy reserves during aggressive interactions, especially in the case of males for territory defense and access to females during the reproductive season. On the other hand, their subterranean niche reduces seasonal environmental fluctuations (e.g., temperature, photoperiod; [5,14]), imposing a situation that contrasts with that of other studied free-living mammals inhabiting above-ground environments. In addition, this species can be successfully maintained in captivity in our laboratory where the responses of the HPA can be monitored during the acclimation process and the effects of chronic and acute stress can be studied under controlled conditions.

Here, we report the results of samplings and experiments performed in the field and the laboratory aimed at contributing to the abovementioned knowledge gaps with regard to the ecophysiology of HPA axis in wild animals and *Ctenomys* in particular. The specific goals were (i) to evaluate seasonal variations in cortisol and corticosterone in free-living male tuco-tucos throughout the breeding cycle, (ii) compare the responses of cortisol and corticosterone to acute stressors under field and laboratory conditions (iii) evaluate the responses of both GCs to captivity and (iv) assess if seasonal changes in GC levels in free-living animals indicate changes in stress levels or reflect fluctuations of basal concentrations with the use of leukocyte profiles.

2. Materials and methods

2.1. Species characteristics

A total of 97 adult males of *C. talarum* were live-trapped in Mar de Cobo (Buenos Aires Province, Argentina; 37°45′S, 57°26′W). *C. talarum* is a medium-sized rodent (adults are 120–220 g) that inhabits individual galleries systems parallel to the surface in southern parts of South America [2,34]. This species is solitary and highly territorial; individuals do not share burrows, except in the case of females and their offspring until dispersal and when mating occurs [13]. It is a highly-aggressive species as evidenced by the presence of scars in captured individuals and the wounds produced in aggressive encounters can be very severe [50]. Tuco-tucos perform most of their activities in their burrows, but they venture away short distances from burrow openings to collect aerial portions of vegetation that they later consume belowground. The species presents a polygynous mating system in which some males monopolize the access to multiple females [48,50]. The natural breeding season extends over nine months starting in late autumn (June, [13,19,28]) and, since pregnancy extends over 95 days [49], most births occur during spring. Their high level of polygyny and high male–male aggression levels represents a scenario for high local mating competition where the spatial location of males represents an important advantage to obtain matings [48]. Both evidences from studies in the wild (skewed adult sex ratio towards females, spatial pattern of sexes, presence of wounds resulted from fights) and captive conditions [13,50] allow us to propose that male–male competition is an important component for mate acquisition.

2.2. Cortisol and corticosterone variations in the field

To assess GC variations in the field throughout the year we captured individuals in three different stages during 2007: (1) non-reproductive season (NRS: April–May, $n = 12$), (2) beginning of the reproductive season (BRS: June–July, $n = 14$) and (3) peak of the reproductive season (PRS: October, $n = 13$, [13,19,28]). The distinction among seasons was made according to the reproductive cycle of females, since males after attaining reproductive maturity do not undergo regression of their testes and contain sperm in their epididymes year round [28]. All samplings were conducted between 10:00 AM and 15:30 PM so that blood samples were obtained within a restricted range of hours of the GC circadian rhythm [4]. However, it is important to note that *C. talarum* individuals showed arrhythmic activity patterns during day and night [27]. Also, in each sampling date we visited a different area of our study population to avoid sampling an individual more than one time. Animals were caught using plastic, tube-shaped live traps (10 cm diameter, 35 cm length) set at fresh surface mounds. These traps have a false floor that triggers the closing of the entrance when an animal steps on it. Holes (20–40 cm depth) were dug in order to access the galleries and the traps were placed as a prolongation of the tunnels. Because traps were monitored closely, animals did not remain in them for more than 20 min (range <1–20 min). After we detected that an individual has been captured (a wire that is tied to the traps' door is not seen anymore from outside when the door has closed), we took the animal in its trap to our van located nearby where blood extractions were performed. Blood samples (500–900 μL) were obtained from the suborbital sinus after 20–30 s of anesthesia with chloroform, using a syringe fitted with a flexible plastic tube which was connected to a heparinized micro-capillary tube. Blood sampling (including previous anesthetization) did not take more than 3 min to guarantee that GC levels were not affected by the extraction procedure. For most species studied, GCs start to increase 3 min after the perception of a stressor, so that samples collected within this period are considered to reflect pre-stress concentrations [35]. In addition, usually 1–2 min more passed between the detection of captures and beginning of blood sampling. However, no correlation was observed between cortisol levels and manipulation time in tuco-tucos that were handled for 1–8 min, indicating that these animals do not consistently increase cortisol in response to handling within this time window (Vera, unpublished data). In other caviomorph rodents (such as guinea pig and yellow-toothed cavy) GC concentrations did not change within a time span of 5 min in response to a stressor [25]. Blood samples were transported to the laboratory in a refrigerated cooler and centrifuged 15 min at 660*g*. The plasma was separated from cells and stored at −20 °C until analysis. All samples were analyzed for cortisol and the ones with enough residual volume ($n = 7$, 8 and 11 for NRS, BRS and PRS, respectively) were further analyzed for corticosterone.

It is known that trapping can elicit increases in plasma GC concentration in a variety of mammals [9,31,38], though this is not always the case [30]. To assess the effects of trapping we conducted an additional sampling during April–May 2008 (i.e., NRS 2008) using a modified trapping technique in which traps were constantly monitored to obtain GC baseline levels (within 3 min) and compare them with delayed samples. To guarantee that blood samples were obtained within 3 min of capture, we set only 6–8 traps per trapper (the three authors and a lab member) and stayed in their proximities to allow a constant monitoring of the traps. As