Seasonal variations of basal cortisol and high stress response to captivity in *Octodon degus*, a mammalian model species

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\textit{Abstract}

Across vertebrates, the hypothalamic–pituitary–adrenal axis is a conserved neuroendocrine network that responds to changing environments and involves the release of glucocorticoids (GCs) into the blood. Few studies have been carried out concerning mammalian adrenal regulation in wild species either in the laboratory or field, and even fewer have been able to determine true glucocorticoid baselines. We studied the South-American caviomorph rodent *Octodon degus*, a diurnal and social mammal that has become an important species in the biological research. First, we determined the plasma cortisol baseline and the acute stress concentrations during the non-reproductive and mating seasons in free-living individuals. Second, using the same protocol we assessed the impact of long-term captivity on the adrenal function in wild-caught degus and degus born in laboratory. Third, we examined laboratory groups formed with degus taken from two distant natural populations; one of them originally occurs at the Andes Mountains in high altitude conditions. The data revealed seasonal modulation of basal cortisol in the wild associated with mating. In laboratory, degus presented higher cortisol stress responses, with greater magnitudes shown in degus born and reared in captivity. No differences between populations were found. The results suggest differential regulatory mechanisms between basal and stress-induced cortisol levels, and context dependence of cortisol modulation in a mammalian species.

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1. Introduction

The vertebrate adrenocortical response to acute stress is a highly conserved physiological process common to all vertebrates and involves the release of glucocorticoids (GCs) into the blood. This “stress response” is controlled by the hypothalamo–pituitary–adrenal (HPA) axis, which is a hormonal communication network sensitive to environmental perturbations (Sapolsky et al., 2000; Wingfield and Sapolsky, 2003). The HPA axis is activated when the hypothalamus is stimulated and secretes arginine, vasotocin, and corticotropin releasing factor to regulate pituitary release of adrenocorticotropic hormone (ACTH), which stimulates the synthesis and secretion of GCs by the adrenal gland. The GCs (cortisol and corticosterone) are steroid hormones with pleiotropic actions, exerting multiple effects from embryonic development and through adult life (Fowden et al., 2006; Seckl, 2004). Among other functions GCs adjust and maintain homeostasis and energy balance by regulating gluconeogenesis, glucose use, and fat and protein metabolism (Cole and Mollard, 2007; Reeder and Kramer, 2005; Sapolsky et al., 2000).

Variations in the energetic demands of animals occur seasonally, paralleling seasonal changes in basal GCs blood concentration (Wingfield, 2005). On the other hand many stressful events are unpredictable and followed by an acute elevation of GCs above basal levels (Sapolsky et al., 2000). Therefore, plasma baseline levels of GCs indicate the daily and seasonal energetic demands in an animal, and stress-induced levels of GCs represent the intensity of the stress response and the sensitivity to adverse events (Wingfield et al., 1998). Seasonal changes in overall adrenocortical function throughout the course of the year have been documented in several free-range animals (Kenagy et al., 1999; Romero et al., 2008; Vera et al., 2011). However, little data are available for baseline and stress-induced GC levels in mammals as compared with data collected for other wild species. The acute increase of GCs concentrations as a result of capture and human handling constitutes a good method for estimating the magnitude of the stress response. The true baseline concentrations can only be obtained by collecting plasma immediately after capture (Kenagy and Place, 2000; Reeder et al., 2004; Romero et al., 2008). Chronic plasma GC elevation because of continuous exposure to stress involves deleterious...
consequences like impaired resistance to disease, infertility, neurological damage and atrophy of body tissues (McEwen and Wingfield, 2003; McEwen, 2000; Romero and Wikelski, 2001; Sapolsky and Pulsinelli, 1985). Under laboratory settings, animals can be exposed to both persistent stressors and consecutive acute stress due to captivity conditions themselves (confined, reduced retreat space, abnormal social groups and monitoring procedures). Profound effects of captivity on the function of the HPA axis have been described and can persist for generations (Matthews and Phillips, 2010; Romero and Wingfield, 1999). This aspect is particularly important because it emphasizes the caution that must be used when extrapolating biological captivity data to natural conditions (Calisi and Bentley, 2009; Kunzi and Sachser, 1999; Marra et al., 1995).

It is hypothesized that GCs are mediators that balance internal physiological dynamics with external environmental conditions (Romero, 2004; Sapolsky et al., 2000). Habitat characteristics and climatological variables influence the physiological stress of animals (Bauer et al., 2013; Breuner et al., 2003; Busch et al., 2011; Mueller et al., 2007; Wingfield et al., 2008). The particular harsh conditions existing at high altitude regions, such as, a higher degree of seasonality, longer winter seasons, lower temperatures, lower partial pressure of oxygen, and lower atmospheric pressure, among others, might be challenging for the organism's homeostasis. Accordingly, previous studies demonstrated that populations occurring at high altitude sites express differences in the sensitivity of their adrenocortical responses (Addis et al., 2011; Beehner and McCann, 2008; Li et al., 2008, 2011; Pereyra and Wingfield, 2003; Sheriff et al., 2012). Although these findings have been essential to elucidate how vertebrates deal with high altitude conditions, the cause that originates differences between populations remains unclear. In general, divergences in the adrenocortical activity between populations could appear because of: (i) physiological adjustments to the prevailing environmental conditions, (ii) differences in developmental processes, (iii) or different genetic backgrounds. Experiments conducted in common garden conditions can be useful to disentangle this assumption (Angelier et al., 2011; Dahl et al., 2012; Dunlap and Wingfield, 1995). Exploring the origin of population's differences in the HPG axis activity can broaden our notion about the adaptive nature of GCs release. As far as we know, common garden experiments addressing divergences in the adrenocortical responses have never been done in wild mammals.

In the present work the study subject is the degu (Octodon degus), a diurnal, social, and endemic caviomorph rodent (~180 g), which occupies a wide distribution throughout north-central Chile. Degus are noted as seasonal breeders typically mating in late autumn (Fulk, 1976). Because of its diurnal behavior, social system, and physiological characteristics, the degu is a species that has become increasingly important in different research fields, including ecology (Ebensperger et al., 2012; Vasquez et al., 2002), animal behavior (Vasquez et al., 2006; Villavicencio et al., 2009), ecosphysiology (Bozinovic et al., 2004, 2009), chronobiology (Mohawk et al., 2005; Vivanco et al., 2007), neurobiology (Helmke et al., 2009; Suarez and Mpodozis, 2009), cognitive sciences (Abraham and Gruss, 2010; Popovic et al., 2010), and Alzheimer and Atherosclerosis research (Homan et al., 2010; Inestrosa et al., 2005). Just like guinea pigs (Hennessey et al., 1995) and humans (Gunnar and Donzella, 2002), the principal measurable plasma GC of degus is cortisol (Gruss et al., 2006; Kenagy et al., 1999). Despite the information available about degus, the modulation patterns of their HPA axis are not well described. Previous studies on plasma cortisol levels have suggested seasonal (Kenagy et al., 1999) and environmental-dependent responses (Bauer et al., 2013; Soto-Gamboa et al., 2005). The impact that the long-term laboratory housing has on the HPA function of degus is not clear. In general, the glucocorticoids responses have been reported for a variety of vertebrate taxa, but remain unknown for most mammals.

We investigated the concentration of plasma cortisol at basal levels and during the stress response. We assessed variations in the magnitudes of cortisol elevation in: (1) Free living individuals of one natural population during two different life history stages, the “non-reproductive season” and the “mating season”. During the mating period degus typically show a strong increase in the agonistic interactions with high social instability (Soto-Gamboa et al., 2005). Hence, our first aim was to assess how seasonal demands affect HPA axis regulation, and also to obtain a parameter of stress responsiveness under natural condition. (2) One laboratory group of captive-wild degus and one group of first generation individuals raised in a laboratory. Degus were related to the same natural population studied in the first aim. (3) Laboratory degus from a different high altitude population. This population occurs in the Andes Mountains and is geographically separated from the population studied in the previous aims. In the same way, we used one group of captive-wild degus and one group of first generation individuals raised in laboratory. The laboratory groups of the two populations described were maintained under the same controlled conditions for 1 year and were measured only at the non-reproductive state (i.e. neutral physiological state). We tested whether the plasma cortisol profiles differ between populations when individuals are held in identical laboratory conditions for 1 year. And also, when individuals from both populations were born and grown for 1 year at the same condition. We experimentally controlled for the influence of environment by conducting a common garden experiment.

We present these results in an effort to establish a plasma cortisol profile in degus as a physiological parameter under natural and captivity contexts. We expect this work to contribute to a broader understanding of mammalian cortisol modulation and its link to behavioral ecology, biomedicine, and animal welfare.

2. Materials and methods

2.1. Subject

The degu is an endemic caviomorph rodent of central Chile with a unique evolutionary lineage, long life, and manageable body size. Moreover they are diurnal, highly social, and relative easy to care for in captivity. Because of these and other characteristics, degus have become an important experimental model that can be bred for many generations (Lee, 2004).

2.2. Free living animals

We investigated a typical natural population of degus in central Chile, Rinconada de Maipú (33°29’S, 70°53’W, 480 m a.s.l.) a field station of the Universidad de Chile located 30 km south-west from Santiago. This population is situated in the Chilean “matorral” zone characterized by marked seasonality with hot and dry summers and cool and moist winters (Fulk, 1976; Vasquez, 1997; Vasquez et al., 2002). In order to assess the seasonal variation of the plasma cortisol baseline, one adult group of six males and five females was caught and sampled during the summer (non reproductive stage) during the first 2 weeks March 2007, and another adult group of five males and nine females was sampled in fall (mating season) during the last week of May and the first week of June 2007. We used 80 Sherman live traps with a grid structure that allowed us to look inside. All traps were located along frequently traveled paths of degus and were within 30 m radius. We were positioned at a concealed location for constant monitoring, so that, we could hear the degus being caught, and could remove them immediately.
The first blood sample was taken within 2 min of capture. Blood was collected from the suborbital sinus into one or two microhematocrit tubes, then sealed at one end and placed on ice until centrifugation. After the first blood sample collection degus were marked with numbered ear tags (National Band & Tag Co.), sexed, weighed and the length of the body was measured. Furthermore, to determine the acute adrenal response to capture and handling, the same individuals were held in the trap and periodically bled twice more. So that, approximately 20 μL of blood sample was withdrawn at zero, 30 and 60 min after capture. Once the samples were obtained degus were released. Each animal was identified with their ear tags, and sampled only once per each season. Finally, in order to estimate the magnitude of the expressed stress response we calculated the difference between the basal level and the highest cortisol concentration reached by each degu.

2.3. Laboratory animals

Animals were maintained under captivity in laboratory conditions during 1 year in groups of 4–6 individuals, separated by sexes, in standard metal cages (80 × 40 × 35 cm) with wood shavings and under near-natural controlled temperature and photoperiod conditions. Food pellets (Champion®) and water were offered ad libitum. We used the same bleeding technique described for the field study, collecting blood samples at 0, 30 and 60 min. Each degu was grabbed and removed from their cages for the collection of the first blood sample. The first blood sample was collected within 2 min of the initial human approach. After the first blood sample collection, degus were equally marked, sexed, weighted, measured and then put inside a Sherman live trap located next to their cages. In between of each bleeding moment, the degus were maintained inside the traps simulating the procedure performed in the field study. We sampled degus belonging to two populations, one of them was the same natural population used for the field study. The second population occurs in a very different habitat, located at high altitude in the Andes mountain range (30°45′S, 70°15′W) at 2600 m a.s.l. (Ebensperger et al., 2012; Quispe et al., 2009). The groups of animals brought to captivity consisted of five males and five females from the lowland population and six males and four females from the high altitude population. All of them had 1 year of acclimation in laboratory after brought from the wild. In addition, we assessed the cortisol modulation of degus born and kept (raised) for 1 year under captivity, but with parents belonging to the two populations studied. The groups of animals raised in laboratory were five males and five females related to the lowland population and five males and five females related with the highland population. All the laboratory animals included in the study (captive-wild and raised in lab) were sampled only during the non-reproductive stage, beginning in March 2007. The mating season was not included as a variable for laboratory animals. Degus in natural populations are highly gregarious mammals with complex social interactions (particularly during mating). We considered this “mating context” not replicable in captivity. The magnitudes of the cortisol stress responses of each individual were calculated in the same manner described with the free-living degus.

2.4. Cortisol assays

The collected blood samples were stored on ice in a cooler, for no more than 5 h after being taken. Then the samples were transported to the laboratory for centrifugation at 7000 rpm for 10 min. Plasma was removed and stored at −20 °C and later transported frozen to University of Washington in Seattle (USA) for hormone analyses. The cortisol levels were measured using radioimmunoassay kit with I125 produced by MP Biomedical. All determinations were run in duplicates. This was a solid phase radioimmunoassay, meaning that the tubes coated with a cortisol antibody are used for the separation of the bound cortisol from the free cortisol. The plasma cortisol competes with cortisol tracer for the limited number of available antibody binding sites thereby reducing the amount of tracer bound to antibody. After an incubation period in a water bath at 37 ± 1 °C for 45 min, the bound and free fractions are separated and the radioactivity quantified. Cortisol concentrations were calculated from gamma counts per minute using the software “RIAzap”. The concentration of cortisol serum is determined by interpolation from a Standard Curve of % of Trace Level vs μg/dL cortisol.

2.5. Statistical analysis

Changes in cortisol levels over time and seasonal differences in baseline and stress-induced glucocorticoid levels were compared with repeated-measures ANOVA after Log transformation. For the field-study, the reproductive stage was used as factor. For the comparisons between field and captive groups, we used origin as factor (free-living, captive-wild or raised in laboratory). And for the laboratory study we used population (lowland or highland) and developmental origin (captive-wild or raised in laboratory) as factors. When significant differences were detected we performed Tukey as post hoc test. The correlation between body mass and sex on the baseline was analyzed with a non-parametric Spearman correlation. The magnitude of the response were obtained by calculating the difference between the lowest and the highest cortisol levels of the individual stress response We compared the magnitudes between groups using one-way ANOVA with season as factor for free-living animals, we used origin as factor for the field/captive analysis. For the laboratory experiment we used two-way ANOVA with population and developmental origin as factors. All analyses were performed using SPSS 13.0.

3. Results

3.1. Field study

Degus demonstrated a significant induced adrenal response to capture and handling ($F_{(1,42)} = 65.295$, $p < 0.001$). We found a significant effect of season ($F_{(1,21)} = 19.989$, $p = 0.002$, see Fig. 1) with higher plasma cortisol concentration in the basal levels and in the 60 min time point during mating. No significant effects were found in the magnitudes of the response ($F_{(1,21)} = 0.053$, $p = 0.342$). There was no correlation between the baseline and body mass (Spearman: correlation coefficient = 0.258, $p = 0.223$) neither to sex (Spearman correlation coefficient, $r_s = 0.023$, $p = 0.913$).

3.2. Laboratory/field study

We analyzed baseline cortisol concentrations and induced stress response among free-living animals, captive-wild and lab-reared individuals from the same population (all during non-reproductive season). All the animals showed and induced adrenal stress response. We found significant differences in the stress response levels between groups being lower for free-living degus ($F_{(1,27)} = 11.465$, $p < 0.001$) (see Fig. 2). No differences were found among cortisol baselines. There were no significant effects for the magnitudes of the stress response ($F_{(1,27)} = 1.141$, $p = 0.341$).

3.3. Laboratory study

Degus demonstrate an induced adrenal stress response ($F_{(1,72)} = 69.911$, $p < 0.001$). They did not show differences in the
stress response between populations ($F_{(1,36)} = 0.012, p = 0.91$). There was a significant effect of developmental origin in the acute stress response ($F_{(1,36)} = 5.963, p = 0.01965$), being higher in animals raised in laboratory (see Fig. 3). There were no differences between populations and developmental origin for baseline levels. We found a significant effect of developmental origin in the magnitudes of the stress responses, being higher in degus raised in laboratory ($F_{(1,36)} = 5.253, p = 0.028$) (see Fig. 4). There was no correlation between the baseline and body mass (Spearman: correlation coefficient = 0.119, $p = 0.464$) and as well no correlation between baseline and sex (Spearman: correlation coefficient = 0.075, $p = 0.647$).

4. Discussion

4.1. Field study

Free-living wild degus showed seasonal modulation of basal plasma cortisol. This observed dynamic suggests an essential role of cortisol in adjusting the overall metabolism throughout different life history stages. We found basal concentrations of plasma cortisol three times higher during mating (Fig. 1), which might be associated with the reproductive social interactions (Goymann and Wingfield, 2004). Social conflicts are an important source of stress in mammals that can be reflected by elevated circulating GC concentrations (Sapolsky, 1992). Indeed, degus are noted as highly social mammals (Ebensperger et al., 2009; Villavicencio et al., 2009) with a period of great social instability during the mating
season when males are competing for females and defending territories, and therefore expressing intense agonistic interactions of dominance among individuals (Soto-Gamboa et al., 2005). Our results match well with that situation because higher basal plasma cortisol was observed during the period with the highest social challenges. Despite the fact that the agonist interactions in degus during mating have been only described in males, our field results showed no differences in cortisol baseline between sexes. It is important to point out that, to our knowledge, there are two previous studies that have described seasonal modulation of plasma cortisol in wild degus, where higher cortisol levels than those shown here were identified during mating season (Kenagy et al., 1999; Soto-Gamboa et al., 2005). However, this difference might be due to different sampling time protocols since we are showing here baseline cortisol levels. In another recent study, wild degus were sampled for basal cortisol plasma levels during the late autumn season (Bauer et al., 2013). Relatively higher baseline levels were obtained in comparison to our early autumn data, suggesting that the baseline can continue increasing towards the winter season (Bauer et al., 2013). Relatively higher baseline levels were sampled for basal cortisol plasma levels during the late autumn season (Bauer et al., 2013). Relatively higher baseline levels were obtained in comparison to our early autumn data, suggesting that the baseline can continue increasing towards the winter season.

Obtaining cortisol baseline levels of degus (and mammals in general) in the field involves several practical difficulties. However, the results presented here encourage continuing the study of cortisol in wild degus, where higher cortisol levels than those shown here were identified during mating season (Kenagy et al., 1999; Soto-Gamboa et al., 2005). However, this difference might be due to different sampling time protocols since we are showing here baseline cortisol levels. In another recent study, wild degus were sampled for basal cortisol plasma levels during the late autumn season (Bauer et al., 2013). Relatively higher baseline levels were obtained in comparison to our early autumn data, suggesting that the baseline can continue increasing towards the winter season (Bauer et al., 2013). Relatively higher baseline levels were obtained in comparison to our early autumn data, suggesting that the baseline can continue increasing towards the winter season.
response, rather it is a reduced reaction to one specific event. Thus, the low basal cortisol levels showed by captive degus might be masking a stressed condition through habituation attained after an extended period of captivity (1 year). The habituation of basal cortisol in captive degus could be being revealed by facilitation of their acute stress response, since they expressed higher cortisol levels after stress induction. In fact, facilitation is commonly used as a diagnostic test for identifying hormonal habituation (Cyr and Romero, 2009).

4.3. Laboratory study

Variations in social structure and behavioral patterns in degus populations have been described in the wild (Ebensperger et al., 2012) and in the laboratory (Quispe et al., 2009). Differences in GCs plasma levels have been also found in correlation with differences in habitat characteristics (Bauer et al., 2013). We included in the study one high altitude population of degus maintained under controlled laboratory conditions. This high altitude population occurs at 2600 (m a.s.l.), experiences contrasting environmental conditions and is geographically isolated. We explored for inter-population variation of the adrenocortical responses under common garden conditions, after 1 year of acclimatization in laboratory. We found no effect of population, neither in cortisol baseline levels nor in the acute stress responses. This suggests no intrinsic differences between populations. However, we ignore the degree of genetic differentiation between the two degu populations studied.

We found a significant effect of the developmental origin in the acute stress response, with no significance on the basal levels. The degus with life experiences exclusively in captivity (raised in laboratory) had higher concentrations in the acute cortisol response no matter the population of origin. Moreover, degus raised in laboratory presented a greater magnitude of the stress response as well (Fig. 4), suggesting that, besides the high cortisol concentration reached, they indeed reacted more strongly to the stressful event (human manipulation). Inter-individual variations in plasma cortisol levels has been described as genetically determined, being also heritable (Bartels et al., 2003; Fedorenko et al., 2004; Solberg et al., 2006). Nevertheless, significant variation can result from developmental plasticity of the HPA axis (Denver, 2009; Meaney et al., 1985). There is accumulating evidence that early stress experiences in mammals can induce changes in thyroid and serotonin (5-HT) activity within the hippocampus (Mitchell et al., 1990; Smythe et al., 1994). These findings propose a pathway whereby an increased 5-HT turnover in hippocampal neurons initiates a signaling cascade that, through epigenetic events, increases GCs receptor transcription in the hippocampus and enhances HPA negative-feedback following stress (Meaney et al., 1993, 2000). This mechanism appears as crucial for the long term effects of early life experiences on the stress reactivity (Champagne, 2013). Previous experiments carried out in degus demonstrated that early life stress experiences caused by parents deprivation interfere with the development of the serotonergic system (Jezierski et al., 2006) and the HPA axis (Becker et al., 2007; Gruss et al., 2006). These studies support the assumption that the developmental environment in degus can affect the regulation of cortisol releasing later in adulthood.

5. Conclusions

In summary, we determined the baseline and acute stress levels of cortisol in plasma of wild and captive degus. Furthermore, we report (1) seasonal variations of the basal levels in the wild. Theses seasonal variations observed correspond with behavioral and social changes that occur during the mating season. The present work confirms that degus undergo marked seasonal dynamics of cortisol releasing. We demonstrate (2) that laboratory conditions affect the magnitude of the stress response of degus, even after a long period of acclimatization. These effects were still greater in degus reared in laboratory conditions. The population of origin does not appear as affecting the cortisol response in our study. Together our results show differential regulatory mechanisms between basal and acute cortisol levels, and context dependence in the modulation of the plasma levels in accordance to seasonal demands, social interactions and laboratory captivity. The results also denote that captivity has an effect on the function of the HPA axis, emphasizing the caution that must be used to interpret laboratory data of non-domesticated mammals.

Given the biomedical and ecological importance of knowing GC physiology in mammals, and the lack of data in non-domesticated species, the present study should contribute with a broader perspective on the nature of this mechanism. Moreover, degus are widely used in field and laboratory research as models for biological phenomena that cannot be studied in other animals. Recognizing how and why GCs release is modulated in this species is fundamental to the general understanding of the endocrinology and the stress response in mammals.

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References


