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Research paper

Seasonal changes in acute stressor-mediated plasma glucocorticoid regulation in New World flying squirrels

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ABSTRACT

Southern flying squirrels have higher circulating cortisol levels than most vertebrates. However, regulation of tissue exposure to cortisol by the hormone's carrier protein, corticosteroid-binding globulin (CBG), appears to be altered due to lower-than-expected CBG expression levels, and a reduced affinity for cortisol. To assess the capacity of flying squirrels to regulate acute stress-mediated cortisol levels, we used the dexamethasone (DEX) suppression test followed by the adrenocorticotropic hormone (ACTH) stimulation test in both the breeding and non-breeding seasons, and quantified resultant changes in plasma cortisol and relative CBG levels. Regulation of cortisol via negative feedback, and the acute stress response appeared to function as they do in other vertebrates during the breeding season, but response to DEX in the non-breeding season showed that the sensitivity of the negative feedback mechanism changed across seasons. The relatively high concentrations of DEX required to induce negative feedback suggests that southern flying squirrels have a reduced sensitivity to cortisol compared with other vertebrates, and that high circulating cortisol levels may be required to compensate for low target tissue responsiveness in this species. Cortisol, but not CBG levels, were higher during the non-breeding than breeding season, and females had higher cortisol and CBG levels than males. Our data suggest that flying squirrel cortisol levels are regulated by negative feedback at a higher set point than in related species. Seasonal changes in cortisol levels, target tissue sensitivity to DEX, and in the capacity to respond to stressors appear to be part of the underlying physiology of southern flying squirrels, and may be required to maximize fitness in the face of tradeoffs between survival and reproduction.

1. Introduction

Glucocorticoids (GC) are important to vertebrates because they mediate physiological responses to environmental stressors, including predators and unpredictable weather events (Sapolsky, 2002; Sapolsky et al., 2000). GCs are regulated in three ways. The first involves the production and release of corticosteroid-binding globulin (CBG; the carrier protein for circulating GCs) from the liver into the circulation, which, in most vertebrates, binds GCs with high affinity and low capacity (Westphal, 1983). CBG's main function is to regulate circulating GC levels and target tissue activation of the glucocorticoid receptors (GR) throughout the body (Breuner and Orchinik, 2002; Rosner, 1990; Schoech et al., 2013). CBG thereby provides (1) a buffer to use of GCs by the tissues when required (e.g., Mendel, 1992), (2) a reservoir of GCs to be released to the tissues over time when required (e.g., Malisch and Breuner, 2010), and (3) transportation of hydrophobic GCs to target tissue receptors that are intracellular or located at the surface of cell membranes (e.g., Hammond et al., 1990), or through intracellular expression of CBG itself (e.g., Mopert et al., 2006). The second way GCs are regulated is through the catabolism of the hormones by the liver, which results in excretion of catabolites through the urine and feces, and different species exhibit species-specific GC clearance rates (e.g., Palme et al., 2005). Finally, GCs are also regulated via the negative feedback mechanism of the hypothalamic-pituitary-adrenal (HPA) axis, or the stress axis. During a stress response, GC levels become elevated above basal levels to help the body deal with the stressor by causing a suite of transient physiological changes via GRs. Once the stressor subsides and there is no longer stimulation of the stress axis, the excess GCs bind to both GRs and mineralocorticoid receptors (MR) in the hippocampus, and to GRs in the hypothalamus and pituitary in saturation. This acts to shut down further release of corticotropin-releasing hormone (CRH) from the hypothalamus, and

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adrenocorticotropic hormone (ACTH) from the pituitary, thereby abolishing the stressor-mediated production and release of cortisol from the adrenal glands (De Kloet et al., 1999; Sapolsky et al., 2000).

The northern (*Glaucomys sabrinus*) and the southern (*G. volans*) flying squirrel have high cortisol levels (the main GC in these species), but only low proportions of this hormone are bound to CBG, and thus, presumably high levels of free GCs are present in circulation (Desantis et al., 2013, 2016, 2018). In flying squirrels, CBG's affinity for cortisol appears to be reduced, which means they lack the regulatory, protective, and cellular activation properties of the protein exhibited by most species (Desantis et al., 2013). The mechanism by which flying squirrels regulate the high baseline levels of circulating cortisol is unclear, but the binding activity between their tissue receptors and cortisol may also be reduced to compensate for the lack of CBG binding capacity. For example, a small number of New World monkeys also have high cortisol and low CBG binding capacity. While their tissue receptors appear to have a normal affinity for cortisol, they exhibit an overexpression of the FKBP51 immunophilin, which inhibits the ability of their GRs to bind cortisol effectively (Scammell et al., 2001). As a result, these New World monkeys are considered glucocorticoid resistant (Chrousos et al., 1982; Scammell et al., 2001). Reduced GC binding activity by receptors allows tissues to more effectively regulate activation by cortisol, because binding to their GRs and MRs is only likely to occur when the receptors are flooded by the hormone (Chrousos et al., 1982; De Kloet et al., 1997; Scammell et al., 2001). GRs and MRs in flying squirrels may have undergone an alteration whereby the affinity of the receptors themselves was reduced directly, or alterations of related molecular phenomenon similar to that in the New World monkeys have occurred, either of which may explain the need for their high basal cortisol levels.

In the first part of our study, we asked whether the sensitivity of the negative feedback mechanism in southern flying squirrels is adjusted to help regulate their circulating cortisol levels. In other words, since the regulatory and protective CBG buffer and reservoir appear mismatched to the high GC levels (Desantis et al., 2013, 2018), how are their tissues protected and how is GC use regulated? To assess the sensitivity and function of the negative feedback mechanism, we challenged individuals with dexamethasone (DEX); a synthetic glucocorticoid. The dexamethasone suppression test allows us to investigate the strength of the negative feedback mechanism by exogenous injection of this synthetic steroid analog (e.g., Boonstra et al., 1998; Chrousos et al., 1982; Kalin et al., 1981; Romero et al., 2008; Sheriff et al., 2011; Taymans et al., 1997). In a healthy individual, the exogenous levels of cortisol (DEX) above a certain threshold will suppress the release ACTH from the pituitary, leading to a negative feedback inhibition of the production and release of cortisol from the adrenal cortex (Cole et al., 2000). An effective test should cause circulating cortisol levels to fall to zero or almost zero (e.g., Boonstra and McColl, 2000; Kalin et al., 1981; Sheriff et al., 2011). In our first experiment, we used the dexamethasone suppression test in southern flying squirrels to test whether the negative feedback regulation of cortisol may be affected. Given what is known about their stress physiology thus far, we hypothesized that the sensitivity of flying squirrels to DEX, and thus presumably to cortisol (at least by the GRs and MRs involved with negative feedback at the level of the brain), has been reduced over evolutionary time. As a result, we predicted that a higher dosage of DEX would be required to invoke negative feedback in southern flying squirrels than in related species.

In the second part of our study, we tested for seasonal and sex-specific variation in the regulation of GCs, and in the acute responses to stress. Vertebrates transition through several biological seasons on an annual basis (e.g., breeding, molt, and overwinter survival). Such transitions are mediated by changes in hormone levels, CBG levels, and in the sensitivity of target tissue receptors for these hormones (e.g., Boonstra et al., 2001a; Delehanty and Boonstra, 2012; Romero et al., 2008). We therefore asked how GC concentrations and CBG levels in southern flying squirrels change seasonally in the context of possible changes in their target tissue responsiveness to cortisol within the negative feedback mechanism, and how their acute response to stress might change seasonally when compared with phylogenetically related species. We assessed seasonal effects using a hormone challenge, which consisted of two parts. The first was a dexamethasone suppression test, as described above, and an ACTH stimulation test, which measures the capacity of the adrenal glands to up-regulate production and release of cortisol in response to ACTH (e.g., Boonstra et al., 1998; Sapolsky, 1983). We measured cortisol concentrations and relative CBG levels across time for the hormone challenge, and across seasons and sexes.

2. Materials and methods

2.1. Study species

Southern flying squirrels are nocturnal, arboreal rodents that are active year-round. Weights range from 46–85 g, and typical home range size is 1.6–2.5 ha (Dolan and Carter, 1977; Bendel and Gates, 1987). Population densities range from 2–5 animals/ha to as many as 12 animals/ha (Dolan and Carter, 1977). Longevity is typically 2–4 years, and they are prey for a variety of species (Dolan and Carter, 1977). In more northern populations, two mating bouts are observed; the first peak occurs in March and April and the second is in July and August (Dolan and Carter, 1977), but a single extended breeding period occurs in the far south from late summer through winter (Stapp and Mautz, 1991). Gestation lasts approximately 40 days, with average litter sizes of 3–4 young being born throughout late April and May, and late August and September, with earlier parturition occurring farther south. Young are weaned within 6–8 weeks and may remain with the female if a second litter is not produced (Dolan and Carter, 1977).

2.2. Study sites & live-trapping

The study site was located near Mississagua Lake in the Kawartha Lakes Region of Ontario, Canada (44°41′18″N/78°20′8″W). Live trapping of southern flying squirrels was conducted in a portion of contiguous forest just west of Kawartha Highlands Provincial Park on the southern edge of the Canadian Shield.

Tomahawk live-traps (Model 102, Tomahawk Live Trap Company, Tomahawk, WI, USA) were used to capture southern flying squirrels and were baited with peanut butter and shelled sunflower seeds. Thirty traps, spaced 20–30 m apart along a trap-line, were fastened with bungee cords to wooden platforms that were mounted on tree trunks approximately 2 m above the ground. Traps were set just prior to dusk and checked 1–2 h after dark, which is a commonly used approach for small mammal trapping (e.g., Boonstra and McColl, 2000; Fletcher and Boonstra, 2006; LaPointe et al., 2015). Our trapping protocols were standardized such that the methods of trap checking, animal transport and handling, and blood sampling were held constant among trapping sessions, thus eliminating confounding variables in the interpretation of GC concentrations.

2.3. Blood sampling

Each evening, all captured animals were transported to Trent University's field research property, the James McLean Oliver Ecological Centre (44°33′50.77 N/78°30′16.11 W; approximately 22 km from the field site), for processing. Animals were kept in live-traps and each was placed inside a pillowcase to help reduce the stress of handling and transportation. Typically, 2–4 animals (maximum = 5) were processed each night. When processing was complete, animals were transported back to the trap line and released at the site of capture.

For all blood-sampling, squirrels were anesthetized with isoflurane (Abbott Laboratories, Montreal, QC, Canada) and blood was then drawn from the sub-orbital sinus (50–200 μL per sample) with heparinized Pasteur pipettes. Samples were stored in 0.6-ml Eppendorf vials and kept on ice until centrifugation, approximately 5–8 h later (depending