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Research paper

Modulation of adrenal steroidogenesis by testosterone in the lizard, *Coleonyx elegans*

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ABSTRACT

Our previous work with adrenocortical cells from several *Sceloporus* lizard species suggests that gonadal hormones influence the steroidogenic capacity and the sensitivity to ACTH. However, there are discrepancies in these cellular response parameters suggesting that the effects of gonadal hormones on adrenocortical function vary with species, sex, age, season, and environmental/experimental conditions. To gain further insight into these complex interactions, here we report studies on *Coleonyx elegans*, Eublepharidae (Yucatán Banded Gecko), which is only distantly related to *Sceloporus* lizards via a basal common ancestor and in captivity, reproduces throughout the year. We hypothesized that a more constant reproductive pattern would result in less variable effects of gonadal hormones on adrenocortical function. Reproductively mature male geckos were orchiectomized with and without replacement of testosterone (300 μg) via an implanted Silastic® tube. Reproductively mature intact female geckos received implants with and without testosterone. After 11 weeks, adrenocortical cells were isolated from these lizards and incubated with corticotropin (ACTH) for 3 h at 28 °C. Three adrenocortical steroids, progesterone, corticosterone and aldosterone, were measured by highly specific radioimmunoassays. The production rate of each steroid was statistically analyzed using established software and net maximal rate (by subtracting the basal rate) in response to ACTH was determined. In general, corticosterone predominated and comprised ~83% of the total net maximal rate, followed by progesterone (~14%) and aldosterone (~3%). Compared to the functional responses of adrenocortical cells derived from other lizards thus far, adrenocortical cells from *C. elegans* exhibited a depressed steroid response to ACTH and this depressed response was more pronounced in male cells. In addition, other sex differences in cellular response were apparent. In female cells, the net maximal rates of progesterone, corticosterone and aldosterone were, respectively, 161, 122 and 900% greater than those in intact-male cells. In contrast, cellular sensitivity to ACTH, as determined by the half-maximally effective steroidogenic concentration (EC_{50}) of ACTH, did not differ between intact-male and intact-female adrenocortical cells. Treatment effects were most striking for corticosterone, the putative, major glucocorticoid in lizards. Orchiectomy caused an increase in the net maximal corticosterone rate equivalent to that of intact-female cells. Testosterone maintenance in orchiectomized lizards completely suppressed the stimulatory effect of orchiectomy. However, orchiectomy with or without testosterone maintenance did not alter cellular sensitivity to ACTH. The effect of testosterone supplementation in intact females, although suppressive, was notably different from its effect in orchiectomized males. Its effect on the net maximal corticosterone rate was relatively modest and did not completely "masculinize" the greater rate seen in intact-female cells. However, testosterone supplementation dramatically suppressed the basal corticosterone rate (by 82%) and enhanced the overall cellular sensitivity to ACTH by 150%, two effects not seen in cells derived from testosterone-treated orchiectomized lizards. Collectively, these findings clearly indicating that the gonad directly or indirectly regulates lizard

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adrenocortical cell function. Whereas other gonadal or extra-gonadal factors may play a role, testosterone appears to be an essential determinant of the observed sex differences in adrenocortical function.

1. Introduction

The glucocorticoid corticosterone plays an essential role in energy intake and partitioning in many vertebrates (Blas, 2015; Cornelius et al., 2013; Landys et al., 2006; Wingfield, 2013). Its circulating levels are determined by a number of factors. Firstly, the hypothalamo-pituitary-adrenal axis (HPA axis), mainly the secretion of adrenocorticotropin (ACTH) from the pituitary gland, is a dominant regulator of circulating corticosterone, given the deleterious effects of HPA axis interruption exhibited in several vertebrate models. However, intraglandular processes, corticosterone inactivation and regeneration and even synthesis at the tissue level (Wyrwoll et al., 2011; Lattin et al., 2016), circulating corticosterone-binding proteins and corticosterone clearance also play a role, albeit, their importance and mechanisms of regulation are poorly understood. Equally poorly understood are the regulation of cognate membrane and intracellular receptors and receptor isomorphs (reviewed in Carsia, 2015) and the myriad factors regulating tissue responses to corticosterone signaling (Lattin et al., 2015, 2016).

Despite the complexity of the role of corticosterone in energy homeostasis, it is not surprising that other homeostatic and behavioral components of a vertebrate life cycle, which require optimal circulating levels of corticosterone for glucose partitioning toward those components, have their respective controls impact on the regulation of circulating corticosterone. The best studied example is that of the hypothalamo-pituitary-gonadal (HPG) axis in that variations in adrenal glucocorticoid output are associated with reproductive maturation and annual breeding cycles (Greenberg and Wingfield, 1987; McQuillan et al., 2003; Romero, 2002; Viau, 2002). Obviously, the various reproductive strategies deployed in vertebrates place demands on energy partitioning. Indeed, the magnitude of the impact of a reproductive strategy on the HPA axis is linked to whether a vertebrate species is short-lived or long-lived. For example, a long-lived species undergoing stress during peak reproductive season may forgo reproduction for survival—an option that is less accessible to a short-lived species. In a long-lived species, stress levels of glucocorticoids may shut down both physiologic and behavioral components of reproduction (Heidinger et al., 2008; Moore et al., 2001; Moore and Jessop, 2003; Rivier and Rivest, 1991; Sapolsky et al., 2000; Tilbrook et al., 2000; Wingfield et al., 1998). In contrast, there are numerous examples in which corticosteroid stress response in short-lived vertebrates is curtailed to favor energy partitioning to reproduction (see Wingfield and Sapolsky, 2003).

The impact of the gonads on the HPA axis appear to be mediated by the end-product sex steroids, testosterone and estrogens. (Carsia et al., 1987b, 2008a, 2008b; Handa et al., 1994; Klukowski, 2011; Klukowski et al., 1997; Pottinger et al., 1996; Young et al., 1996). Whether end-product sex steroids act as positive or negative modulators of circulating corticosterone is unclear given the number of contradictory studies across vertebrates studied, but the trend suggests that estrogens are stimulatory and androgens are inhibitory (Handa et al., 1994). Obviously, more vertebrate models are required because the complexities of the life cycles and life history stages of various vertebrate taxa provide challenges to forming some consensus regarding the mechanisms regulating the interaction of the HPA and HPG axes (Viau, 2002).

In this context, lizards may be suitable models to study the interplay and cost-benefit trade-offs between adrenocortical stress response and reproductive success/survival (see Anderson et al., 2014). In lizards, the major circulating glucocorticoid is corticosterone. Members of this ectothermic group have long-lived and short-lived life cycles, exhibit a diverse range of physiological (e.g., oviparity/viviparity; gonochoristic/parthenogenetic) and behavioral reproductive strategies (polygyny/monogamy; polyandry/monandry) (Wade, 2011) and have breeding opportunities ranging from nearly continuous to seasonally restrictive (Lofts, 1978; Vitt and Caldwell, 2014). Unfortunately, the few studies involving orchiectomized lizards have produced disparate conclusions (see Klukowski, 2011). Nevertheless, with regard to end-product sex steroids, the body of evidence from work with lizards suggests that testosterone has a negative impact on the HPA axis (Carsia et al., 2008a, 2008b; Klukowski, 2011) whereas estrogens for the most part are stimulatory (Cartledge and Jones, 2007).

Testosterone has been investigated more broadly than other sex steroids in lizards because of its role in territorial defense and other male reproductive functions (Golinski et al., 2011; Sinervo and Miles, 2011). Although testosterone may act at all levels of the HPA axis in lizards, work thus far suggests that its main action is to curtail the release of ACTH. Indeed, a recent study with the relatively short-lived, polygynous species, *Sceloporus undulatus*, supports the negative action of testosterone on the proximal HPA axis (Klukowski, 2011). By contrast, our work with adrenal steroidogenic (adrenocortical) cells isolated from several *Sceloporus* lizard species broadly suggests that the negative modulation of testosterone on the HPA axis also culminates in altered adrenocortical function at the cellular level (Carsia et al., 2008a, 2008b).

Adrenocortical cells derived from several *Sceloporus* lizard species exhibit either modest or substantial sex-dependent seasonal, environmental and stressor-related changes in functional properties (Carsia and John-Alder 2003; Carsia et al., 2008a, 2008b; Carsia et al., 2012). Our results indicate differences in adrenal steroidogenic function in terms of steroidogenic capacity and cellular sensitivity to ACTH. For example, female cells tend to exhibit greater steroidogenic capacity and sensitivity to ACTH. These sex differences are at least partially dependent on gonadal status (Carsia et al., 2008a, 2008b). Overall, ovariectomy tends to "masculinize" the properties of female cells, that is, to reduce steroidogenic capacity and cellular sensitivity to ACTH and orchiectomy tends to "feminize" male cells by raising steroidogenic capacity and cellular sensitivity to ACTH. Furthermore, our work suggests that in males, testosterone is the main testicular factor mediating these effects on adrenocortical cells (Carsia et al., 2008a, 2008b).

Although there is a tendency for reproductive manipulations to affect adrenocortical function, the degree to which a functional modality is affected (e.g., sensitivity to ACTH and/or steroidogenic capacity) is inconsistent. We have hypothesized (Carsia et al., 2008b) that this lability of functional response of adrenocortical cells derived from different lizard species to reproductive manipulations is due to supervening factors derived from complex interactions of sex and reproductive patterns with environmental and experimental conditions (e.g., laboratory-housed vs. field-active). It is further plausible that the sequential regression and recrudescence of gonadal function accompanying seasonal breeding contribute to this lability of adrenal response to reproductive manipulations. We reasoned therefore, that the use of a lizard spe-