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Non-breeding gonadal testosterone production of male and female Northern Cardinals (*Cardinalis cardinalis*) following GnRH challenge

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Abstract

Yearly, testosterone (T) levels fluctuate as many vertebrates cycle through reproductive and non-reproductive periods. Among many temperate birds, it is well established that levels of T peak as gonads recrudesce for breeding and then fall as gonads regress prior to the non-breeding season. While the tissues producing breeding season T are well studied, the tissues responsible for non-breeding T have received less investigative attention. We examined the ability of male and female Northern Cardinals (*Cardinalis cardinalis*) to elevate gonadal T following standardized injections of gonadotropin-releasing hormone (GnRH) across three non-breeding seasons. Males and females were capable of significantly elevating gonadal T production following GnRH injections during periods of reproductive quiescence. The magnitude of T elevation varied across the non-breeding season, but not between sexes. To our knowledge, this is the first report of a significant increase in gonadal T production following GnRH injections administered in the non-breeding season.

Highlights

► We examined if cardinals can elevate gonadal testosterone within the nonbreeding period. ► Both sexes were administered injections of gonadotropin-releasing hormone (GnRH). ► Both sexes could elevate gonadal testosterone after GnRH injections in the nonbreeding season.

Introduction

Vertebrates must modify aspects of their behavior, physiology, and morphology to coordinate life history stages with predictable changes in environmental conditions [41], [42], [67]. In birds that reproduce seasonally, such phenotypic adjustments are often mediated by collapse of the reproductive axis and subsequent gonadal recrudescence [17], [38], [39]. Breeding events require recrudescence of the reproductive axis, during which the hypothalamus increases secretion of gonadotropin-releasing hormone (GnRH) that stimulates the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary, resulting in gonadal growth and increased steroid hormone production (especially testosterone, T). Termination of breeding is initiated by the collapse of the reproductive axis, resulting in decreased GnRH levels, gonadal regression, and a drastic reduction in T secretion. However, T production in many species does not cease during reproductive quiescence [1].

Many avian species have detectable levels of T during the non-breeding period (e.g., male European nuthatches, *Sitta europaea* [35]; male downy woodpeckers, *Picoides pubescens* [29]; male red grouse, *Lagopus lagopus scoticus* [45]; male and female Northern Cardinals, *Cardinalis cardinalis* [25]; male and female spotted antbirds, *Hylophylax n. naevioides* [20]; male and female song sparrows, *Melospiza melodia* [12], [66]; and male and female European robins, *Erithacus rubecula* [33], [50]. Additionally, numerous studies have examined relationships between T and its metabolites and winter territorial behavior (e.g., [12], [20], [29],

[33], [35], [45], [50], [53], [54], [66]). Yet, quantifying the tissue/tissues responsible for T secretion within the non-breeding season has received little attention.

Gonadotropin releasing hormone challenges are bioassays commonly used to determine the reproductive status of individuals by assessing the gonad's ability to produce T [8], [16], [22], [34], [43], [44], [48], [56], [63], [65]. Individuals are given either an intravenous or intramuscular injection of GnRH to stimulate the release of LH from the pituitary, resulting in the secretion of T from the gonads if the gonads are capable of responding to LH stimulation [24]. Concentrations of T before and after the GnRH injection are compared to assess gonadal responsiveness. Because these injections initiate a hormonal pathway unique to the hypothalamic–pituitary-gonadal (HPG) axis, these injections do not stimulate T secretion from any other tissue capable of producing the hormone (e.g., brain, adipose tissue, and adrenals). Consequently, the GnRH challenge is an effective technique to determine if any circulating T in the non-breeding period is gonadal in origin.

Here, we examine if the Northern Cardinal (*C. cardinalis*; hereafter, "cardinal") produces gonadal T during the non-breeding season. Cardinals are year-round resident birds ranging from Central America to Southern Canada [18]. Unlike many temperate species, breeding pairs display year-round territoriality ([18], [23], M.S. DeVries, pers.obs.). Further, both males and females sing [68], provide approximately equal rates of parental care [18], and readily display intraspecific and interspecific aggression during

territorial defense ([23], M.S. DeVries, unpubl.data). In addition, the annual T profiles of male and female cardinals fluctuate little throughout the year and are nearly identical in pattern [25]. These annual profiles further indicate that both sexes are capable of producing T levels during the non-breeding period (approximately 1–1.5 ng/ml) that are not drastically lower than T concentrations characteristic of the breeding season (approximately 1.5–2 ng/ml), when the gonads are fully functional [25]. Such a unique pattern of annual T production suggests that the gonad could be contributing to the relatively high levels of non-breeding T seen in both sexes.

To determine whether non-breeding T is gonadal in origin in male and female cardinals, we assessed gonadal T production of this species using standardized injections of GnRH during three non-breeding seasons defined as the months of December through the following March, 2008–2009, 2009–2010, 2010–2011. We predicted that the gonads of male and female cardinals would elevate T secretion following GnRH challenges within the non-breeding period (December, January, February, and March) because of the individual variation in T levels seen during prior repeated sampling of individuals across these months. Further, we predicted that T response to GnRH challenges would strengthen as the breeding season approached due to gonadal recrudescence.

Section snippets

General field techniques

This study was conducted in Hattiesburg, Mississippi, USA at the Eubanks/Lake Thoreau (ELT-USM) property owned by the University of Southern Mississippi (USM). Behavioral and physiological attributes of this population are assessed year-round, with non-breeding investigations performed during December, January, February, and March. Although both sexes engage in territorial behavior (e.g., song and aggression) in this population as early as December, breeding does not begin until April. All…

Development and controls

Initial tests of GnRH injections performed in February 2008 did indicate significant T elevation following both dosages of GnRH (paired samples *t*-tests: 1.25 μg dosage, $t=4.47$, df=5, $p=0.007$; 2.5 μg dosage, $t=6.35$, df=5, $p=0.001$). We proceeded with GnRH injections using the 2.5 μg dosage considering it elicited a greater average T response (2.5 μg dose, mean=1.41 ng/ml; 1.25 μg dose, mean=0.90 ng/ml). Initial and post-challenge T levels of control birds injected with PBS in March 2008 did not…

Gonadal response to GnRH challenge during the non-breeding period

Prior investigations examining gonadal activity following GnRH challenges within non-breeding seasons report a lack of significant T elevation during this period (e.g., Hungarian white geese, *Anser domesticus* [22]; European ground squirrel, *Spermophilus citellus* [43]). Such results are not surprising considering that T production is usually assumed to be from non-gonadal tissue (e.g., adrenals, brain, adipose tissue, etc. that should not respond to GnRH or LH) within non-reproductive periods…

Conclusions

We conclude that male and female cardinals are capable of producing gonadal T following GnRH challenge within the non-breeding months. In addition, the magnitude of T response following GnRH injections is very similar between the sexes during this period. This study will hopefully foster additional examinations to determine if other species have the ability to produce gonadal T during the non-breeding season. Further, our results indicate that relationships between T and non-breeding season…

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