General and Comparative Endocrinology 178 (2012) 306–313

Contents lists available at SciVerse ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Effects of food restriction on steroidogenesis in dispersed adrenocortical cells from Yarrow's Spiny Lizard (*Sceloporus jarrovii*)

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ARTICLE INFO

Article history:
Received 18 September 2011
Revised 27 May 2012
Accepted 1 June 2012
Available online 18 June 2012

Keywords:
Adrenocortical cells
Adrenocortical stress response
Corticosteroids
Food restriction
Lizards
Reptilian

ABSTRACT

Changes in energy balance can lead to functional alterations at all levels of the hypothalamic–pituitary–adrenal (HPA) axis. However, relatively little is known about how energy balance affects functional properties of adrenocortical cells themselves. We investigated effects of restricted food intake on sensitivity to ACTH and rates of steroidogenesis in adrenocortical cells isolated from growing female and male Yarrow's Spiny Lizards (*Sceloporus jarrovii*). At the end of the feeding regimen, we assayed acute (3 h) progesterone (P_4), corticosterone (B), and aldosterone (ALDO) production in response to ACTH in dispersed adrenocortical cells. Food restriction depressed growth rate by about 50% in both males and females but did not alter baseline plasma B measured at 10 weeks in either sex. At the cellular level, food restriction had the following effects: (1) increased basal B production in both sexes and basal ALDO production in males, (2) increased net maximal rates of production of P_4, B, and ALDO in response to ACTH, and (3) no overall effect on adrenocortical cellular sensitivity to ACTH. There were modest sex differences: overall rates of P_4 production were 46% greater in cells from females than from males, and in response to food restriction, the net maximal rate of ALDO production was 50% greater in cells from males than from females. Our results demonstrate that food restriction in *S. jarrovii* increases adrenocortical cellular rates of steroid production without affecting overall cellular sensitivity to ACTH.

1. Introduction

Adrenocortical function plays a central role in vertebrate intermediate metabolism, and nutrition has a profound impact on the hypothalamic–pituitary–adrenal (HPA) axis in both mammalian and non-mammalian species. In laboratory rats (*Rattus norvegicus*), food intake has been shown experimentally to influence circadian periodicity in plasma corticosterone [35], and a similar response may occur in marine iguanas (*Amblyrhynchus cristatus*) in association with foraging [54]. Furthermore, in free-living animals, requirements for energy mobilization have been invoked as the reason for seasonal variation in glucocorticoid output of the HPA axis [42]. Studies on the laboratory rat indicate that the activity of the HPA axis is part of a larger homeostatic system that regulates caloric flow [1,21,22,23,32,41,49,57]. In the guinea pig (*Cavia porcellus*), even brief periods of maternal caloric restriction can imprint abnormal HPA axial function that is expressed in adult offspring [36]. Functional lability involves both the proximal portion of the HPA axis (hypothalamus and pituitary) as well as the distal portion (adrenal cortex). With regard to the adrenal cortex itself, the increase in adrenocortical steroidogenesis by caloric restriction is thought to be mediated in part by alterations in circulating leptin and the activity of adrenal innervation [21].

In birds, evidence suggests an interaction between hormonal regulation of energy (fat) stores and the regulation of the HPA axis. Furthermore, in controlled studies, food restriction of sufficient duration typically increases plasma concentrations of corticosterone (B) [6,37,43,53]. Less information is available for ectothermic vertebrates, but the existing findings on the impact of food restriction on interrenal/adrenal steroidogenic function are varied. In tadpoles, for example, steroidogenic functions of the hypothalamic–pituitary–interrenal (HPI) axis are increased by food restriction, reflecting the need to mobilize tail energy stores for development and metamorphosis [19,30]. However, in juvenile frogs, food restriction does not appear to alter interrenal steroidogenic function because, without sizable energy stores, it is thought that increases in circulating corticosteroids would be deleterious to energy balance [19,20]. By contrast, food restriction in fish appears to increase interrenal steroidogenesis [38].

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http://dx.doi.org/10.1016/j.ygcen.2012.06.002

Most previous research has focused on the proximal portion of the HPA axis (i.e., hypothalamic–pituitary responses). By contrast, effects of food restriction on the adrenal gland itself have largely been neglected [21]. Studies on species of domestic fowl indicate that dietary protein restriction increases adrenal steroidogenic function at the cellular level in chickens (*Gallus gallus domesticus*) [11,13,14,40,51] and has the opposite effect in turkeys (*Meleagris gallopavo*) [8,12]. It follows that investigations of lability in adrenocortical cellular functions are likely to be informative and variable in other species. While investigations exploring the impact of caloric restriction on avian species are numerous, similar studies on non-avian reptiles – including the species-rich and ecologically diverse Squamata (lizards and snakes) – are lacking. Such information can help to bridge the gap in knowledge between non-mammalian endothermic vertebrates (i.e., birds) and ectothermic vertebrates (non-avian reptiles, amphibians and fish), especially since birds are evolutionarily derived reptiles. Furthermore, an understanding of how the reptilian HPA axis is modulated by stress, including nutritional stressors, will help to clarify comparative questions of functional variation among species [42]. Representative species of Squamata can be particularly opportune for this purpose because of their ecological diversity.

In the present contribution, we report the influence of food restriction on adrenal steroidogenic (adrenocortical) cell function in Yarrow's Spiny Lizard (*Sceloporus jarrovii*). We used dispersed adrenocortical cells harvested from lizards that had been involved in an investigation of the influence of food restriction on energy allocation to growth and body composition in lizards [17]. Dispersed adrenocortical cell preparations have been used to help characterized adrenal functional lability in response to stress in avian species [8,10,11,12,14,40,51], and we have previously validated this approach to investigate components of adrenal stress response in lizards [7].

In our previous study of seasonal variation in adrenocortical cellular function in field-active *Sceloporus undulatus* [7], we reported substantial decreases in cellular sensitivity to ACTH and maximal rates of ACTH-induced B production from the breeding season in early spring to the post-breeding period of mid-summer, accompanied by a deterioration in body condition. These results suggest that food restriction may cause reductions in steroidogenic capacities and sensitivity to ACTH, a prediction that we test in the present study using adrenocortical cells from *S. jarrovii*. However, any such prediction is rather tenuous, given differences between species of lizards, their natural habitats, and experimental conditions. Furthermore, adrenocortical responses to dietary manipulation have previously been shown to differ between chickens and turkeys even under very similar experimental conditions (see above). Thus, the alternative prediction, that food restriction will cause an increase in cellular steroidogenic capacities in *S. jarrovii*, as in domesticated turkeys [8,12], is almost equally plausible based on current evidence.

As part of our assessment of cellular functions, we report P_4 in addition to B and ALDO production. The emerging picture is that adrenal P_4 plays significant gestational [29] and behavioral roles in lizards [33,52]. In several species of lizards, P_4 is a significant secretory product in *in vitro* adrenal preparations [24,25,29,31,33]. Furthermore, our previous work with dispersed adrenocortical cells from *Sceloporus* lizards has demonstrated modest to robust P_4 production in response to ACTH [7,9].

2. Materials and methods

2.1. Animals

All experimental procedures were reviewed and approved by the Rutgers University Animal Care and Facilities Committee (protocol #01-019). As reported previously [17], male and female *S. jarrovii* yearlings (2–3 months old) were collected in September, 2004 near Buena Vista Peak in the Chiricahua Mountains, Coronado National Forest, Arizona, USA (31°54–55′N, 109°16′W). Animals were collected under permit from the Arizona Game and Fish Department (SP 553889) and housed at Rutgers under permit from the New Jersey Division of Fish and Wildlife (SH 25086). In the laboratory, lizards were held individually in plastic cages ($36 \times 42 \times 46$ cm) containing a bedding of sand and two bricks that were stacked to form a shelter and basking site. Water was always available in a shallow dish lined with aquarium gravel. Lizards were housed on a 12:12 L:D photoperiod with an incandescent light as a heat source on a 10-h thermal period. Temperatures within cages varied along a gradient from 25 to 45 °C during photophase and averaged 19 °C during scotophase. Several criteria, including plasma B, indicate that these captivity conditions after an acclimation period were not stressful [16,17,18].

Animals were acclimated to captivity for 1 week and were then assigned to one of four size-matched treatment groups: high-food males ($n = 11$) and females ($n = 8$), and low-food males ($n = 10$) and females ($n = 12$). These four groups did not differ in initial snout-vent length (SVL) ($F_{3,37} = 0.82$; $P = 0.49$) or body mass ($F_{3,37} = 0.24$; $P = 0.87$) prior to food manipulation. For 10 weeks, high-food groups were provided three crickets (*Acheta domestica*) per day, while low-food groups were provided one cricket/d. Each week, all live and dead crickets that remained in each cage were counted, and food consumption (crickets/d) for each animal was estimated by assuming that all missing crickets had been consumed. The high-food ration of three crickets per day allowed lizards to feed *ad libitum* [17] in that lizards under this feeding regimen had demonstrably not eaten all the crickets offered. Thus, groups fed three crickets per day are the unrestricted, *ad libitum*-feeding controls. Hereafter, the feeding groups are designated "*ad libitum*-fed" and "food-restricted". Snout-vent length (mm) and body mass (g) were measured at biweekly intervals, and growth rate in SVL (mm/d) and body mass (g/d) were estimated as the slope of the linear regression of body size (SVL or mass) on elapsed time (d) for each individual lizard [16]. Growth was linear over the duration of the 70-d experimental period, as assumed by this method. Furthermore, the food-restriction group continued to grow albeit more slowly than the *ad libitum*-fed controls (see Section 3). Thus, the food-restriction regimen was not starvation.

At the conclusion of the experiment, animals were euthanized by decapitation, blood was collected, and carcasses were necropsied for adrenal glands. Blood for determination of plasma B was collected in heparinized microhematocrit capillary tubes from the open neck wound. Blood samples were centrifuged, and plasma fractions were collected in capped microfuge tubes and held at −20 °C until thawed for radioimmunoassay. Adrenal glands were immersed and stored in ice-cold basic medium (see below) containing bovine serum albumin (1 mg/ml; Fraction V; Sigma Chemical Co., St. Louis, MO) until processing for dispersed adrenal cells.

2.2. Preparation of dispersed lizard adrenal cells

Adrenal glands (two per lizard) were harvested from a subset of eight lizards chosen arbitrarily from each feeding group. Adrenocortical cells for incubations were prepared by pooling both adrenal glands from each of two lizards (i.e., 4 adrenal glands). Thus, a total of four independent pooled cell preparations, where each preparation contained cells from two lizards, were analyzed for each feeding group. Each pooled cell preparation was incubated in duplicate under each incubation condition, resulting in a total of eight incubations under each condition for each feeding group.

The basic medium used for adrenal tissue dissociation and dispersed cell incubation was Krebs–Ringer-HEPES buffer containing glucose [24.2 mM HEPES; (*N*-2-hydrdoxyethylpiperazine-*N*′-2-eth-