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Research paper

Effects of exogenous leptin on seasonal reproductive responses to interacting environmental cues in female Siberian hamsters

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ABSTRACT

Animals living in temperate climates respond to environmental cues that signal current and future resource availability to ensure that energy resources are available to support reproduction. Siberian hamsters (*Phodopus sungorus*) undergo robust gonadal regression in short, winter-like photoperiods as well as in response to mild food restriction in intermediate photoperiods. The goal of the present study was to investigate whether leptin is a relevant metabolic signal in regulating gonadal regression in response to diminishing food availability. Adult female hamsters housed in short-day (winter-like) or intermediate (fall-like) photoperiods received either *ad libitum* access to food or mild food restriction (90% of *ad libitum* intake) and were treated with either leptin or a vehicle for five weeks in order to determine the ability of leptin to inhibit gonadal regression. At the end of five weeks, vehicle-treated hamsters showed physiological signs associated with ongoing gonadal regression, such as decreases in body mass and food intake, cessation of estrous cycling, and small decreases in reproductive tissue mass. Leptin did not modify changes in body mass, food intake, hormone concentration, or tissue mass, but showed a tendency to support estrous cycling, particularly in response to food restriction in the intermediate photoperiod treatment. Overall, leptin appears to play a minor role in coordinating reproductive responses to multiple environmental cues, at least in the early stages of gonadal regression.

1. Introduction

Animals living in temperate climates experience extreme fluctuations in resource availability on a seasonal basis that influence survival and reproductive success. All components of reproductive function, from gametogenesis to parental care of offspring, are energetically expensive, particularly for females, and are typically not favored over survival if energy stores are insufficient (Bronson, 1985; Schneider, 2004). Many temperate zone species have thus evolved to restrict reproduction to times of year that are most favorable for survival of self and offspring (i.e., times of maximal energy availability) (Bronson, 1985; Bronson and Heideman, 1994; Goldman, 2001). In order to time reproduction appropriately, animals respond to cues signaling energy availability in the environment, as well as to internal metabolic signals.

Seasonally breeding animals such as Siberian hamsters respond to multiple environmental factors in order to determine the appropriate time for reproduction. Siberian hamsters are reproductively active in long-day (LD), summer-like (>14 h of light per day) photoperiods and undergo robust gonadal regression accompanied by dramatic loss of body mass and decline in food intake in short-day (SD), winter-like (<12 h of light per day) photoperiods (Hoffmann, 1982; Bartness and Wade, 1985; Goldman, 2001). Photoperiod can be used to predict the future status of factors such as precipitation, presence of conspecifics, and perhaps most critical, food availability, and is therefore conceptualized as an "initial predictive" cue, which is used to coordinate broad physiological responses over time (gonadal regression) (Baker, 1938; Wingfield and Farner, 1980). Reproductive timing can then be fine-tuned in response to the immediate status of factors critical to offspring survival (e.g., food availability) acting as "supplementary" cues (Wingfield and Farner, 1980). To mimic the interactions of these two types of environmental cues, we employed a recently established laboratory

Abbreviations: LD, long-day; SD, short-day; ID, intermediate-day; AL, *ad libitum*; FR, food restriction; PWAT, parametrial white adipose tissue.

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paradigm that is useful for exploring how animals respond to interacting signals of energy availability. Male Siberian hamsters housed in constant photoperiods of either greater than 14 h (summer) or less than 10 h (winter) of light per day in laboratory conditions show definitive physiological and behavioral states such that supplementary cues (e.g., food restriction) do not trigger reproductive responses (Paul et al., 2009a,b). However, if male hamsters develop within a constant, intermediate (13.5 h light) photoperiod, supplementary cues (food availability, social housing) can trigger the initiation of gonadal regression through neuroendocrine mechanisms similar to those controlling the response to photoperiod (Paul et al., 2009a,b; Bailey et al., 2017).

Environmental and metabolic signals that modulate reproductive function are integrated by the hypothalamo-pituitary-gonadal (HPG) endocrine axis. The hypothalamic gonadotropin releasing hormone (GnRH) neurons control secretion of the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland, thereby leading to changes in gonadal function. HPG axis activity—specifically, the pulse frequency of GnRH neurons—controls reproductive function and is affected by both initial predictive and supplementary environmental cues as well as metabolic signals of energetic state, a notable example of which is leptin. Leptin is a peptide hormone that is released from white adipose cells, and is a critical signal in body mass homeostasis as well as an array of reproductive functions in both sexes such as the onset of puberty and maintenance of fertility (Cheung et al., 1997; Ahima and Flier, 2000; Fernandez-Fernandez et al., 2006; Quennell et al., 2009; Hausman et al., 2012; Landry et al., 2013; Chehab, 2014; De Bond and Smith, 2014; Pankov, 2015; Perez-Perez et al., 2015). Leptin-deficient animals exhibit infertility associated with a lack of GnRH and LH pulsatile release, delayed puberty, and impaired cycling and ovulation in females, all of which are alleviated through treatment with exogenous leptin (Barash et al., 1996; Chehab et al., 1996; Mounzih et al., 1997; Schneider et al., 1998; Hill et al., 2008; Evans and Anderson, 2012; Luo et al., 2016).

Seasonally breeding animals, in addition to modifying reproductive function, exhibit seasonal changes in a host of physiological parameters, such as body mass, food intake, and immune function (reviewed in Bartness and Wade, 1985; Nelson et al., 2002). Concurrent with these changes, leptin expression and secretion fluctuates on a seasonal basis in response to photoperiod in several seasonally breeding species, such as hamsters, ground squirrels, and sheep (Klingenspor et al., 1996; Boyer et al., 1997; Marie et al., 2001; Bartness et al., 2002; Mercer et al., 2000; Rousseau et al., 2002). The potential role of leptin in regulating seasonal changes in metabolic and reproductive function is currently unclear, due to conflicting reports of the effects of exogenous leptin owing to differences in method of administration. For example, in Siberian hamsters, exogenous administration of leptin has photoperiod-specific effects on changes in body mass and immune function, but does not affect food intake or HPG axis activity within photoperiod treatments in the same way depending on administration (chronic/continuous vs. acute) (Atcha et al., 2000; Klingenspor et al., 2000; Drazen et al., 2001; Bartness et al., 2002; Carlton and Demas, 2014). Administration of leptin via daily injection triggered a decline in food intake in both LD- and SD-housed Siberian hamsters, but leptin administered continuously via mini-osmotic pump had no effect on food intake in similarly housed hamsters (Atcha et al., 2000; Klingenspor et al., 2000). Leptin appears to be an ideal candidate for an endocrine signal of energy availability relevant for seasonal reproductive function; however, chronic, continuous leptin administration is ineffective in stimulating reproductive physiology in SD photoperiod-housed, gonadally regressed male and female Siberian hamsters (Atcha et al., 2000).

The goal of the present experiment was to assess the role of leptin in signaling energetic state to the reproductive axis in environments providing varying photoperiodic and metabolic information relevant to current and future energy availability. To accomplish this, we administered exogenous leptin to female Siberian hamsters exposed to either a SD photoperiod or an intermediate-day (ID) photoperiod, with and without mild (90% of *ad libitum* intake) food restriction. We hypothesized that females given food restriction in an intermediate photoperiod would undergo gonadal regression as males do, and that their reproductive response to this cue is mediated by metabolic signals resulting from decreased food availability, i.e., decreased leptin, rather than from the photoperiod. Therefore, we predicted that providing exogenous leptin in these conditions would prevent gonadal regression, as measured by cessation of estrous cycles, decline of HPG axis activity, and atrophy of reproductive tissues. By comparison, we predicted that providing leptin to females exposed to a short-day photoperiod would not be effective, regardless of food treatment, because photoperiod acts independently of real-time energetic status to stimulate gonadal regression (Atcha et al., 2000).

2. Materials & methods

2.1. Animals & housing

Adult (>60 days of age) female Siberian hamsters (n = 55) were obtained from 25 litters produced by 10 breeding pairs in our intermediate-day photoperiod (13.5:10.5 h light:dark cycle, lights on at 0130 h Eastern Standard Time, EST) breeding colony at Indiana University. Animals were weaned at 18 days of age, and subsequently housed either individually or with one to four same-sex littermates before entering the experiment. All hamsters were individually housed in polypropylene cages ($27.5 \times 17.5 \times 13.0$ cm) with Sani-chip bedding material for one week prior to the start of experimental treatments. Animals received *ad libitum* access to food (Lab Diet 5001, PMI Nutrition) throughout development prior to the experiment, and *ad libitum* access to tap water at all times. Temperature and humidity were maintained at 20 ± 2 °C and 50 ± 10%, respectively. All animal procedures were reviewed and approved by the Indiana University Bloomington Institutional Animal Care and Use Committee.

2.2. Experimental design

Hamsters were randomized by breeding pair source and litter into eight experimental groups, in a full factorial design by photoperiod (intermediate-day [ID] or short-day [SD], 8:16 h light:dark cycle, lights on at 0700 h EST), food availability (*ad libitum*, AL or food restriction, FR), and mini-osmotic pump treatment (recombinant murine leptin [PeproTech Inc., Rocky Hill, NJ, USA] or the vehicle [Tris buffer]). Animals acclimated to individual housing for one week, after which a baseline blood sample was collected and baseline food intake and estrous cycling were monitored for five days (see subsequent subsections for details). Mini-osmotic pump implantation surgery was conducted post-baseline monitoring, after which animals recovered for three days before half of them were transferred to SD photoperiod treatment. Daily food treatment and weekly monitoring of body mass began on the day of photoperiod transfer and continued for five weeks, along with periodic estrous cycle monitoring and blood sampling, at the end of which animals were euthanized for tissue collection at 1000 h EST.

2.3. Mini-osmotic pump implantation

Hamsters were anesthetized with isoflurane and surgically implanted with a mini-osmotic pump subcutaneously in the intra-scapular region (Alzet model 2006: 200 μL volume, 0.15 μL/