Hormones and Behavior 66 (2014) 802–811

Contents lists available at ScienceDirect

Hormones and Behavior

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

Regular article

Leptin mediates seasonal variation in some but not all symptoms of sickness in Siberian hamsters

Elizabeth D. Carlton *, Gregory E. Demas

Department of Biology, Program in Neuroscience and Center for the Integrative Study of Animal Behavior, Indiana University, Bloomington, IN 47405, USA

ARTICLE INFO

Article history:
Received 2 October 2014
Revised 5 November 2014
Accepted 8 November 2014
Available online 14 November 2014

Keywords:
Lipopolysaccharide
Energetics
Seasonality
Ecoimmunology
Infection-induced hypothermia
Infection-induced anorexia

ABSTRACT

Many seasonally breeding species, including Siberian hamsters (*Phodopus sungorus*), exhibit seasonal variation in sickness responses. One hypothesis regarding the mechanism of this variation is that sickness intensity tracks an animal's energetic state, such that sickness is attenuated in the season that an animal has the lowest fat stores. Energetic state may be signaled via leptin, an adipose hormone that provides a signal of fat stores. Siberian hamsters respond to extended housing in short, winter-like days by reducing fat stores and leptin levels, relative to those housed in long, summer-like days. Sickness responses are also attenuated in short-day hamsters as compared to long-day hamsters. We hypothesized that leptin provides a physiological signal by which seasonally breeding animals modulate sickness responses, such that animals with higher leptin levels show increased sickness intensity. To test this, we provided short-day hamsters with a long-day-like leptin signal and assessed their responses to lipopolysaccharide (LPS), a sickness-inducing antigen. We compared these responses to short-day vehicle-, long-day vehicle-, and long-day leptin-treated hamsters. Unexpectedly, LPS induced a hypothermic response (rather than fever) in all groups. Short-day vehicle-treated hamsters exhibited the greatest LPS-induced hypothermia, and leptin treatment attenuated this response, making hypothermia more long-day-like. Contrary to our hypothesis, short-day leptin-treated hamsters showed the least pronounced LPS-induced anorexia among all groups. These results suggest that leptin may mediate some but not all aspects of seasonal sickness variation in this species. Future studies should be targeted at determining roles of other energetic hormones in regulating seasonal sickness response variation.

Introduction

Seasonally breeding animals must respond to temporal changes in environmental factors like climate fluctuations, social interactions, and resource availability. While their abiotic and biotic environments are changing, seasonal breeders respond with appropriate morphological, physiological, and behavioral adaptations in order to maximize their chances of survival and reproductive success (Bronson, 1985). Such seasonal adaptations include changes in reproductive function and behavior, frequency and magnitude of agonistic behaviors, metabolism, and immune function (Demas et al., 2010). All of these processes require substantial energy, and as energetic resources may be less plentiful during certain times of the year (e.g., winter), changes in the expression of these traits occur when energy is shifted away from certain processes and toward those that will prioritize immediate survival (Nelson and Demas, 2004). In particular, immunity is quite sensitive to the energetic state of organism, as seasonal alterations in immune function can be best predicted by changes in an animal's energetic state rather than reproductive state or photoperiodic cues (Demas, 2004). Seasonal changes in immune responses have been documented in all three branches of the immune system (i.e., innate, cell-mediated, humoral) in several different species of mammals and birds (Martin et al., 2008b). Seasonal changes in immunity are commonly observed in inducible immune defenses because the energetic costs of mounting an immune response can be very high (e.g., can raise resting metabolic rate by as much as 50%) (Lochmiller and Deerenberg, 2000).

One of the initial and more energetically expensive immune responses is the acute phase response (APR), and the behavioral and physiological manifestations of sickness that accompany it. During the APR, pro-inflammatory cytokines are released from immune cells and act on the brain to generate the symptoms of a sickness response. Sickness responses are characterized by hyperthermia (i.e., fever) or hypothermia, anorexia, body mass loss, reductions in social, hedonic, and sexual behaviors, and hypothalamic–pituitary–adrenal (HPA) axis stimulation (Hart, 1988; Tizard, 2008). While these symptoms may appear to be a result of infection-induced weakness or malaise, these responses are actually a well-adapted mechanism to aid the host organism in clearance of the infectious agent (Hart, 1988). Blocking fever and anorexia during sickness can actually result in increased mortality via failure to eliminate the infection (Covert and Reynolds, 1977; Kluger

* Corresponding author at: Department of Biology, 1001 E. 3rd Street, Indiana University, Bloomington, IN 47405, USA. Fax: +1 812 855 6705.
E-mail address: elcarlto@indiana.edu (E.D. Carlton).

http://dx.doi.org/10.1016/j.yhbeh.2014.11.003
0018-506X/

et al., 1975; Kyriazakis et al., 1998; Vaughn et al., 1980), while blocking glucocorticoid production can cause mortality via sepsis (Bertini et al., 1988). Mounting an appropriate sickness response is clearly beneficial to an organism's survival, but being sick also carries significant energetic costs that can be detrimental to survival if too severe (Buchanan et al., 2003; Maier et al., 1994; Plata-Salaman, 1996). While variations in sickness response intensity at the extremes of the spectrum clearly negatively affect survival, the ability to modulate sickness intensity between these "mortality endpoints" may be critical for ensuring survival in environments with variable energetic resource availability.

Seasonally breeding animals live in environments where energetic resources vary across the annual cycle (i.e., resources are more plentiful in summer than winter), and studies of sickness responses in several seasonally breeding species have revealed that sickness response intensity can also vary with the seasons (reviewed in Ashley et al., 2012; Ashley and Wingfield, 2012). Collectively, the patterns of sickness response variation in these species reveal that there is not one critical season in which animals display a weak or strong sickness response, suggesting that seasonal photoperiodic cues or reproductive status may not drive variation in sickness intensity. Rather, the common predictor of sickness response intensity across these studies is the current energetic state of the animal—sickness responses are attenuated in the season in which the organism has the lowest energy reserves (i.e., lowest body mass and fat stores) (Bilbo et al., 2002; Owen-Ashley et al., 2006, 2008; Owen-Ashley and Wingfield, 2006; Prendergast et al., 2008). In further support of the hypothesis that energetic state is a predictor of sickness response intensity in seasonally breeding animals, pre-sickness body mass and body fat levels are correlated with infection-induced anorexia and body mass loss, such that animals with higher initial body masses and fat stores show greater percent decreases in food intake and body mass after experimental infection (Owen-Ashley et al., 2006, 2008). These observations suggest that the magnitudes of the energetically expensive components of a sickness response are constrained by a minimum body mass that an animal can reach before it risks its survival (Ashley and Wingfield, 2012; Owen-Ashley and Wingfield, 2007).

If energetic state is the critical predictor of seasonal variation in sickness response intensity, then the adipose hormone leptin is a promising candidate for a neuroendocrine mediator of this variation. Leptin is not only tightly coupled with the energetic state of an organism, but it also interacts with the immune system (Carlton et al., 2012; La Cava and Matarese, 2004). Leptin levels are directly proportional to the mass of adipose tissue in several mammalian species (Johnson et al., 2004; Maffei et al., 1995), and as such, high levels of leptin indicate adequate energy stores, whereas low levels are consistent with energy deficit. Leptin levels change across seasons in seasonally breeding animals, and these seasonal changes track seasonal changes in body mass and body fat (Concannon et al., 2001; Gaspar-Lopez et al., 2009; Horton et al., 2000). Immune function can be restored via leptin treatment in animals that have been food deprived or have had body fat experimentally reduced (Demas and Sakaria, 2005; Lord et al., 1998), and there is also evidence that leptin may modulate seasonal changes in immunity (Drazen et al., 2001). Although there is no yet established role of leptin in mediating seasonal variation in sickness responses, there is considerable evidence that leptin does influence sickness responses (Harden et al., 2006; Sachot et al., 2004), although the direct mechanisms and their effects are not entirely understood (Carlton et al., 2012).

The goal of the present study was to test the hypothesis that leptin serves as a neuroendocrine signal mediating seasonal variation in sickness responses. To accomplish this, we housed male Siberian hamsters (*Phodopus sungorus*) in long and short days to induce two photoperiodic morphs, experimentally elevated leptin levels in a subset of hamsters in each morph, and then measured sickness response variables (e.g., body temperature, anorexia, body mass loss, anhedonia, nest building behavior, HPA axis activation) in response to inoculation with lipopolysaccharide (LPS), a sickness-inducing bacterial mimetic. When housed in short days, Siberian hamsters regress their gonads to a non-reproductive state and decrease food intake, body mass, and fat stores. In addition, Siberian hamsters have lower leptin levels in short days as compared to long days (Horton et al., 2000) and display less intense sickness responses (i.e., lower fever amplitude, shorter durations of and lesser decreases in food intake and body mass loss, lesser decreases in hedonic and nest shredding behaviors, higher cortisol secretion) (Bilbo et al., 2002, 2003; Wen et al., 2007). We predicted that if leptin mediates seasonal variation in sickness responses, then short-day leptin-treated hamsters would display sickness responses similar to long-day vehicle-treated hamsters and would display more intense sickness responses than short-day vehicle-treated hamsters. Leptin treatment, however, should have no effect on the sickness responses of long-day housed hamsters because previous studies in this species have shown that leptin does not enhance other measures of immunity in long-day animals even though it enhances them in short-day animals (Demas, 2002; Drazen et al., 2001).

Methods

Animals and housing conditions

Adult male (>60 days of age) Siberian hamsters (n = 117) were obtained from our breeding colony at Indiana University. The progenitors of these animals were generously provided by Dr. Randy Nelson (Ohio State University) and Dr. Timothy Bartness (Georgia State University). In order to minimize the effects of inbreeding, our animals are outbred approximately every 10 generations. All animals were initially group housed (2–5 with same sex siblings on weaning at 17–18 days of age) in long-day photoperiods (light:dark (L:D) 16:8), and then individually housed in polypropylene cages (27.8 × 17.5 × 13.0 cm) for one week prior to the start of the experiment. Food (Laboratory Rodent Diet 5001, LabDiet, St. Louis, MO, USA) and tap water were available ad libitum during the entire course of the experiment. Temperature (20 ± 2 °C) and humidity (50 ± 10%) were maintained at constant levels. Animals were then randomly assigned to either long (L:D 16:8) (n = 40) or short days (L:D 8:16) (n = 74) for the remainder of the study. A greater number of hamsters were housed in short days to account for reproductive non-responders (described below). All animal methods were reviewed and approved by the Institutional Animal Care and Use Committee at Indiana University Bloomington (protocol no. 10-038).

A subset of hamsters within the short-day group often fails to show reproductive responsiveness to photoperiod (i.e., do not display gonadal regression, reductions in body mass and fat stores, or changes in pelage coloration and thickness) despite prolonged exposure to short days. These individuals are referred to as photoperiodic non-responders (Puchalski and Lynch, 1986). After ten weeks of exposure to short-day photoperiods, 36 animals were determined to be non-responders (defined by a reduction in body mass less than or equal to 10% of their mass at the beginning of the experiment) and were removed from the experiment. At the conclusion of the experiment, paired testes mass was collected to confirm short-day responsiveness (defined as a paired testes mass < 0.15 g) (Greives et al., 2008). At the end of the study, we were left with 40 hamsters exhibiting the long-day phenotype (referred to as LD from here forward) and 38 hamsters exhibiting the short-day responder phenotype (referred to as SD from here forward).

Experimental methods

During the first 10 weeks of photoperiodic treatment, hamsters were weighed weekly to the nearest 0.1 g to track photoperiodic responsiveness. After these ten weeks and when the photoperiodic non-responders were removed from the study, body mass (to the