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Sickness behaviour after lipopolysaccharide treatment in ghrelin deficient mice

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ABSTRACT

Ghrelin is an orexigenic hormone produced mainly by the gastrointestinal system and the brain. Much evidence also indicates a role for ghrelin in sleep and thermoregulation. Further, ghrelin was recently implicated in immune system modulation. Administration of bacterial lipopolysaccharide (LPS) induces fever, anorexia, and increased non-rapid-eye movement sleep (NREMS) and these actions are mediated primarily by proinflammatory cytokines. Ghrelin reduces LPS-induced fever, suppresses circulating levels of proinflammatory cytokines and reduces the severity and mortality of various models of experimental endotoxemia. In the present study, we determined the role of intact ghrelin signaling in LPS-induced sleep, feeding, and thermoregulatory responses in mice. Sleep-wake activity was determined after intraperitoneal, dark onset administration of 0.4, 2 and 10 μg LPS in preproghrelin knockout (KO) and wild-type (WT) mice. In addition, body temperature, motor activity and changes in 24-h food intake and body weight were measured. LPS induced dose-dependent increases in NREMS, and suppressed rapid-eye movement sleep, electroencephalographic slow-wave activity, motor activity, food intake and body weight in both Ppg KO and WT mice. Body temperature changes showed a biphasic pattern with a decrease during the dark period followed by an increase in the light phase. The effects of the low and middle doses of LPS were indistinguishable between the two genotypes. Administration of 10 μg LPS, however, induced significantly larger changes in NREMS and wakefulness amounts, body temperature, food intake and body weight in the Ppg KO mice. These findings support a role for ghrelin as an endogenous modulator of inflammatory responses and a central component of arousal and feeding circuits.

1. Introduction

Activation of the immune system by systemic microbial infection triggers a coordinated set of adaptive behavioral changes collectively called sickness behavior. The acute phase response is characterized by these behavioral adaptations and changes in immune, neurologic, endocrine, metabolic functions including decreased food intake, social withdrawal, lethargy as well as altered sleep-wake activity. Bacterial lipopolysaccharide (LPS), a heat-stable biologically active component of the outer membrane of Gram-negative bacteria, is a well-characterized and widely used model of experimental endotoxemia (reviewed in Alexander and Rietschel, 2001). Peripheral administration of LPS induces a broad range of biological effects such as fever, anorexia, increased non-rapid-eye movement sleep (NREMS) and decreased rapid-eye movement sleep (REMS) in humans (Mullington et al., 2000), rabbits (Krueger et al., 1986; Kimura et al., 1994), rats (Kapás et al., 1998) and mice (Opp and Toth, 1998; Morrow and Opp, 2005; Nadjar et al., 2013). Numerous studies demonstrate that LPS-induced sickness behavior is mediated primarily by proinflammatory cytokines such as interleukin 1 beta (IL1β), IL6, and tumor necrosis factor alpha (TNFα) acting in the brain or on vagal cytokine receptors to modulate brain cytokine expression (reviewed in Krueger et al., 1994; Singh and Jiang, 2004).

Ghrelin is a brain-gut peptide hormone implicated in various physiological processes including food intake and sleep-wake activity [reviewed in Szentirmai and Kapás (2012)]. For example, sleep deprivation increases plasma and hypothalamic ghrelin levels in rats (Bodosi et al., 2004). Intracerebroventricular (icv) and hypothalamic microinjections of ghrelin increase wakefulness, suppress non-rapid-eye-movement sleep (NREMS) and rapid-eye-movement sleep (REMS) in rats (Szentirmai et al., 2006, 2007). Icv injections of ghrelin increase wakefulness and suppress sleep, while systemic administration of the peptide fails to alter sleep-wake activity in mice (Szentirmai, 2012). Ghrelin receptor

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knockout mice exhibit attenuated responses to arousal-promoting stimuli such as fasting and exposure to a new environment (Esposito et al., 2012). Preproghrelin knockout (Ppg KO) mice have slightly altered sleep-wake activity at thermoneutral ambient temperature and normal food anticipatory activity (Szentirmai et al., 2007, 2010) but display severe sleep and thermoregulatory impairments when challenged with fasting in a cold environment (Szentirmai et al., 2009).

Recent evidence indicates a role for ghrelin in modulating the activity of the immune system. Ghrelin and the ghrelin receptor (growth hormone secretagogue receptor, GHS-R) are expressed in human T lymphocytes and monocytes (Dixit et al., 2004). Ghrelin inhibits the expression and production of IL1β, IL6, and TNFα by activated human T cells and monocytes (Dixit et al., 2004). Ghrelin reduces LPS-induced increases in serum levels of TNFα, IL1β and IL6, partially, through the activation of the vagus nerve in rats and mice (Wang et al., 2009; Wu et al., 2007). Ghrelin treatment attenuates LPS-induced fever (Soriano et al., 2011), reduces the severity and mortality of various models of experimental endotoxemia and sepsis (Chang et al., 2003; Chorny et al., 2008; Gonzalez-Rey et al., 2006; Wu et al., 2007). Furthermore, ghrelin improves bacterial clearance from peritoneal fluid *in vivo* and shows bactericidal properties against *Escherichia coli in vitro* (Chorny et al., 2008). Serum ghrelin levels, after LPS treatment, are elevated (Chang et al., 2003; Wang et al., 2009) or suppressed (Basa et al., 2003; Hataya et al., 2003; Wang et al., 2006) depending on the time of the measurement.

In the present study we determined the role of intact ghrelin signaling on LPS-induced sleep, feeding, and thermoregulatory responses in mice. We report that after a high dose of LPS challenge Ppg KO mice display augmented hypothermia and NREMS and suppressed REMS and food intake compared to wild-type (WT) controls. These findings further support a role for ghrelin in sleep-wake activity, thermoregulation and immune responses.

2. Methods

2.1. Animals

Male, 5–6 months old Ppg KO [originally named as Ghrelin−/− mice, (Sun et al., 2003)] and wild-type (WT) mice were used in the experiments. Breeding pairs of ghrelin KO and WT mice with a C57BL6J/129SvEv genetic background, backcrossed to C57BL6J for 10 generations, were generated and given as a generous gift by Drs. Roy G. Smith and Yuxiang Sun at Baylor College of Medicine (Houston, TX), and further bred and kept in a non-SPF animal facility at Washington State University. Each mouse used in the experiments was genotyped (Transnetyx, Cordova, TN). Procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Protocol (ASAF # 3512 and 3948) was approved by the Institutional Animal Care and Use Committee at Washington State University.

2.1.1. Surgery

The body weights of the WT and KO mice at the time of surgery were 32.2 ± 0.6 g and 32.8 ± 0.7 g, respectively. During surgery, mice were anesthetized with intraperitoneal (ip) injection of ketamine-xylazine mixture (87 and 13 mg/kg, respectively). The animals were implanted with cortical electroencephalographic (EEG) electrodes, placed over the frontal and parietal cortices, and electromyographic (EMG) electrodes in the dorsal neck muscles. The EEG and EMG electrodes were connected to a pedestal fixed to the skull with dental cement. Temperature-sensitive transmitters were implanted in the abdominal cavity for telemetry temperature and activity recordings. Mice were allowed to recover from surgery for at least 10 days before baseline recordings started. During the recovery and experimental periods, all mice were housed in individual recording cages located in a sound-attenuated environmental chamber at a constant, thermoneutral ambient temperature (29 ± 1 °C) and controlled light–dark cycles [12–12 h, lights on: Zeitgeber time (ZT0) at 5 am]. Food and water were available *ad libitum* throughout the experiments. The animals were fed with regular lab chow (Harlan Teklad, Product No. 8640); fat, proteins, and carbohydrates provided 17%, 29%, and 54% of calories, respectively.

2.1.2. Experiment

After recovery from surgery, mice were handled daily to habituate them to the experimental procedures. On the baseline day, the animals were injected with isotonic saline (ip, 0.1 ml/10 g body weight). On the test day, LPS from *E. coli* serotype 0111:B4 (Sigma, St. Louis, MO) was dissolved in isotonic NaCl and was injected ip in the same volume. Three doses of LPS, 0.4 ($n = 8$ for Ppg WT, $n = 7$ for Ppg KO), 2 ($n = 7$ for Ppg WT, $n = 8$ for Ppg KO), and 10 μg/mouse ($n = 10$ for Ppg WT, $n = 8$ for Ppg KO) were tested. Separate groups of animals were used for each dose of LPS. All injections were performed 5–10 min prior to the onset of the dark phase. Recordings were carried out for 24 h after the injections.

2.1.3. Sleep-wake recordings and analyses

Recording cables connected the animals to commutators, which were further routed to Grass Model15 Neurodata amplifier system (Grass Instrument Division of Astro-Med, Inc., West Warwick, RI). The high-pass and low-pass filters for EEG signals were 0.5 and 30.0 Hz, respectively. The EMG signals were filtered with low and high cut-off frequencies at 100 and 10,000 Hz, respectively. The outputs from the amplifiers were fed into an analog–digital converter (digitized at 256 Hz) and collected by computer (SleepWave software, Biosoft Studio, Hersey, PA). Sleep-wake states were scored visually off-line in 10-s segments according to the following criteria. Non-rapid-eye-movement sleep (NREMS): high-voltage EEG delta waves (0.5–4 Hz) and decreased muscle tone; rapid-eye-movement sleep (REMS): predominant EEG theta activity (6–8 Hz) and lack of muscle tone with occasional muscle twitches; wakefulness (W): low-voltage EEG activity, and varying levels of increased muscle activities. Time spent in W, NREMS and REMS was calculated in 2- and 12-h blocks. EEG power data from each artifact free 10-s segment were subjected to off-line spectral analysis by fast Fourier transformation. EEG power data in the range of 0.5–4.0 Hz during NREMS were used to compute EEG slow-wave activity (SWA). EEG SWA data were normalized for each animal by using the average EEG SWA across 24 h on the baseline day as 100. The results were averaged in 2-h bins.

2.1.4. Telemetry recordings

Core body temperature and locomotor activity were recorded by Mini Mitter telemetry system (Philips Respironics, Bend, OR). Temperature and activity values were collected every 1 and 10 min, respectively, throughout the experiment and were averaged into 2- and 12-h time blocks. Activity values were normalized for each animal by using the average activity across 24 h on the baseline day as 100.

2.2. Statistics

Time spent in wakefulness, NREMS and REMS, as well as, EEG SWA, body temperature and motor activity were calculated in 2- and 12-h blocks. Three-way mixed ANOVA was performed separately for each dose of LPS across 24 h on the 12-h data (independent measure: genotype, repeated measures: time and treatment). When ANOVA indicated significant effects,