Hormones and Behavior 97 (2018) 56–66

Contents lists available at ScienceDirect

Hormones and Behavior

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

Central ghrelin receptor stimulation modulates sex motivation in male rats in a site dependent manner

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ARTICLE INFO

Keywords:
Ghrelin
Sex motivation
Anticipation
VTA
MPOA
Intracranial drug delivery
Ghrelin receptor antagonist
Ghrelin receptor knock out rats

ABSTRACT

Ghrelin, a hormone produced primarily by the stomach, has been associated with motivational processes that include reward-seeking behaviors. In male laboratory mice, elevation of ghrelin levels enhances some aspects of sexual motivation and behavior, whereas in other experiments with male mice, rats, and other species, ghrelin treatment or food deprivation decreases sexual motivation and/or behavior. The present tested the hypothesis that stimulation of ghrelin receptors in different brain regions have opposite effects on male sexual motivation and behavior. To do this we examined appetitive and consummatory sex behaviors of male rats with a truncated ghrelin receptor (FHH-GHSR$^{m1/Mcwi}$), and that of their WT (FHH) littermates. We also examined the effects of ghrelin or the ghrelin antagonist D-Lys-GHRP6 delivered into the VTA or the MPOA on appetitive and consummatory sex behaviors in male Long Evans rats. Results demonstrate that rats with a truncated ghrelin receptor, or rats that are food deprived, show deficits in anticipatory sex. Furthermore, although ghrelin does not further stimulate sex anticipation in rats when infused into the VTA, intra-VTA infusions of D-Lys-GHRP6 into the VTA further decreases in sex anticipation in food deprived rats. In contrast, ghrelin delivery into the mPOA decreased sex anticipation compared to saline or D-Lys-GHRP6 infused rats. Overall, these data suggest that ghrelin receptor signalling is important for full expression of appetitive sex behaviors. Within the VTA, ghrelin may act to enhance sex motivation, while acting on the mPOA to decrease sex motivation and promote foraging.

1. Introduction

The 28 amino-acid peptide hormone ghrelin is produced mainly in the gastric mucosa of the stomach and circulates throughout the body to regulate food intake, body weight, energy expenditure, and glucose homeostasis (Kojima et al. 1999; Tschop et al. 2000). In laboratory rodents, plasma ghrelin concentrations fluctuate across the light/dark cycle, with ghrelin concentrations peaking at around the onset of the dark phase, when animals eat most of their food (Drazen et al. 2006). Peaks in ghrelin concentrations, however, can be entrained to the time of the day in which meals are available, and are associated with food anticipatory activity (Blum et al. 2009; Cummings et al. 2001; LeSauter et al. 2009). Once feeding begins, ghrelin concentrations drop rapidly, a process associated with satiety (Cummings et al. 2001). Ghrelin's binds to its only known receptor, the growth hormone secretagogue receptor-(GHSR) to generate its biological actions. The GHSR is expressed in a number of tissues including the ovaries, pancreas, stomach, thyroid, and testes (Guan et al. 1997; Howard et al. 1996; Papotti et al. 2000). In addition, the GHSR is expressed in the central nervous system, including in several hypothalamic nuclei associated with the regulation of food intake and energy balance (Guan et al. 1997; Zigman et al. 2006). Ghrelin acts at these sites to stimulate the release of orexigenic peptides such as Neuropeptide Y (NPY) and agouti related peptide (AGRP), and to promote food intake and adiposity (Nakazato et al. 2001; Tschop et al. 2000).

In addition to hypothalamic nuclei, receptors for ghrelin are detected in a number of regions within midbrain and limbic areas that are associated with learning, emotion and motivation processes (Zigman et al. 2006). One of these regions, the ventral tegmental area (VTA), contains relatively abundant expression of GHSRs, that is primarily localized on dopamine cells (Abizaid 2009; Abizaid et al. 2006). Ghrelin administration into the VTA increases action potential frequency, and results in increased dopaminergic release and turnover in the nucleus accumbens (NA) (Abizaid et al. 2006; Jerlhag et al. 2006). Furthermore,

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http://dx.doi.org/10.1016/j.yhbeh.2017.10.012
Received 30 June 2017; Received in revised form 28 September 2017; Accepted 24 October 2017

ghrelin activity within this pathway increases both food intake and food seeking behaviors in response to cues that predict food rewards (Abizaid et al. 2006; Jerlhag et al. 2010; King et al. 2011; Naleid et al. 2005; St-Onge et al. 2016). Moreover, ghrelin receptor antagonists delivered into the VTA attenuate peripheral ghrelin-induced food intake and the intake of food after an overnight fast (Abizaid et al. 2006). Ghrelin also enhances the hedonic value of drugs including alcohol, nicotine, cocaine, amphetamines, and opiates (Abizaid et al. 2011; Davis et al. 2007; Dickson et al. 2011; Tessari et al. 2007; Wellman et al. 2005). Together these data support the notion that ghrelin may promote a state of increased motivation that generalizes to a variety of reinforcers, including sex.

Recent evidence supports a role for ghrelin in sexual motivation. Male mice, given either peripheral injections of ghrelin or infusions of ghrelin into the VTA, showed an increased preference for receptive female mice when given the choice between an estrous female and a male (Egecioglu et al. 2016; Prieto-Garcia et al. 2015). The same treatment also enhanced aspects of consummatory sexual behavior, including a reduction in the latency to mount a receptive female, as well as an increase in the number of and duration of mounts. In addition, mice with targeted deletion of the GHSR (GHSR KO mice) or wild type (WT) mice treated with a GHSR-1a antagonist either peripherally, into the VTA, or into the laterodorsal tegmentum (LTDT) display decreased preference for receptive female mice, as well as decreased mounting and intromitting, behaviors. These behaviors were restored with L-DOPA treatment (Egecioglu et al. 2016; Prieto-Garcia et al. 2015).

In contrast to those data, it is well established that most animals will forgo reproduction when faced with increased energetic demands, or when food is scarce (Schneider 2004; Schneider et al. 2013). In males rats this is reflected by an increased latency to approach receptive females in animals that are food deprived (Caquineau et al. 2012). This effect may be mediated in part by ghrelin given that there is emerging substantial evidence of ghrelin modulating the hypothalamic-pitiuitary-gonadal (HPG) axis inmale and female rodents (Bertoldi et al. 2011; Farkas et al. 2013; Fernandez-Fernandez et al. 2005; Kluge et al. 2009). Furthermore, intraperitoneal injections of ghrelin inhibit mating vocalizations in male mice in response to receptive females and had no effect on the preference for the odour of female bedding (Shah and Nyby 2010). Moreover, sexually naïve rats infused with ghrelin into the third ventricle displayed significantly increased latencies to mounting, intromission, and ejaculation when allowed to interact with a sexually receptive female, and these effects were prevented with ICV treatment with a GHSR receptor antagonist (Babaei-Balderlou and Khazali 2016). Although these discrepancies may in part be due to differences in species or in the operational definition of sexual motivation, they do bring into question the role of ghrelin in modulating male sexual behavior.

To explain these paradoxical effects, we propose that there are independent neural substrates underlying the motivational and consummatory components of sexual behavior (Beach 1976; Everitt 1990), and that ghrelin acts on these in parallel as a means to managing the trade-off between feeding and reproductive function as reviewed recently (Schneider and Deviche 2017). Using this model, one could argue that ghrelin acts on the VTA to stimulate a general state of motivation, while targeting regions associated with reproductive function to inhibit them and bias behavior towards foraging. In this sense, while food deprivation may have an overall effect inhibiting sex behavior to promote feeding, ghrelin infused directly into the VTA should have an overall stimulating effect on sexual motivation and behavior, and antagonists into the VTA may further decrease sex motivation in food deprived animals. In contrast, increased ghrelin signalling in brain regions associated with sexual behaviors may result in decreased sexual motivation and copulatory behaviors as reported in previous studies (Babaei-Balderlou and Khazali 2016; Caquineau et al. 2012; Jarmon and Gerall 1961; Shah and Nyby 2010). One potential target for this suppressive effect is the medial preoptic area (mPOA), a region of the brain important for the integration of sensory and hormonal information, and critical for the elicitation of sexual motivation and consummatory behaviors in males of a number of avian and mammalian species (Will et al. 2014). Importantly, this region contains ghrelin receptors (Zigman et al. 2006), and is a region where other peptide metabolic signals like neuropeptide Y inhibit sex behaviors in male rats (Kalra et al. 1988).

To address these issues, we conducted a series of experiments that examined the role of ghrelin receptor function on sexual behavior. We first evaluated sexual behavior and sexual motivation in fawn hooded hypertensive rats with a point mutation that results in a truncated GHSR protein (FHH-GHSR$^{m1/Mcwi}$), as well as that of their wild type (FHH-WT) littermates. These rats show some minor deficits in some metabolic parameters, reduced responses to stimulants and intracranial self-stimulation (Clifford et al. 2012), as well as a decrease in palatable food intake in a manner similar to GHSR KO mice (MacKay et al. 2016), and as such represent a good model for the study of ghrelin signalling and male sexual behavior. As part of this experiment, we observed the behavior of these rats towards sexually receptive females when they were sexually naïve, and across several subsequent tests to determine whether mutations to the ghrelin receptor also results in alterations in appetitive and consummatory sexual behaviors in sexually experienced animals. In the second experiment, we compared differences in sexual behaviors in sexually experienced rats with free access to food that received ghrelin directly into the VTA with that of rats that had been food deprived and that received a GHSR antagonist delivered onto this region. Finally, we examined the effects of ghrelin receptor stimulation in the mPOA on sexual motivation.

2. Methods

2.1. Experimental subjects

Male Long Evans rats purchased from Charles River farms (St. Constant, Que) and male rats from the FHH-GHSR$^{m1/Mcwi}$ strain and their FHH WT littermates served as subjects in these experiments. The FHH-GHSR$^{m1/Mcwi}$ rats originated from breeding pairs purchased from Transposagen (Lexington, KY) and bred at Carleton University with FHH WT rats obtained from Charles River Farms. In addition, Long Evans female rats weighing 250–270 g were purchased from Charles River Farms (St- Constant, QC) to serve as stimuli animals. Rats weighed between 350 and 450 g at the onset of the study and were housed individually under standard laboratory conditions on a reverse 12 h light dark cycle with lights on at 7:00 PM. Rats had free access to food and tap water, and were exposed to environmental enrichment (i.e. Plexiglas tubes, pumpkin seeds, chew blocks etc). One week after arrival, female rats were bilaterally ovariectomized (OVX) through lumbar incisions under a mixture of 3 parts isoflurane to 1 part oxygen (3:1). Females were treated post-operatively with subcutaneous injections of 3 cm^3 physiological saline, and 1 mg/kg Metacam. These stimulus females were primed with estradiol benzoate (EB; 10 μg/0.1 mL sesame oil) and progesterone (P; 500 μg/0.1 mL sesame oil) administered 48 and 4 h prior to sexual behavior tests respectively. All steroid compounds were obtained from Steraloids (Newport, RI). EB (10 μg) and P (500 μg) and dissolved in 0.1 mL sesame oil under low heat for approximately 30 min, and stored at room temperature. All procedures were approved by the Carleton University Animal Care Committee and followed the guidelines of the Canadian Council for Animal Care.

2.2. Anticipatory and consummatory sex behavior in males

All sexual behavior training and testing occurred in bi-level chambers (Mendelson and Pfaus 1989), during the middle third of the dark cycle. These chambers were designed to facilitate the experimenter's view of the full behavioral repertoire of sexual behaviors (Mendelson