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GHRELIN SIGNALING IN THE VENTRAL TEGMENTAL AREA MEDIATES BOTH REWARD-BASED FEEDING AND FASTING-INDUCED HYPERPHAGIA ON HIGH-FAT DIET

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Abstract—Ghrelin is a potent orexigenic hormone that acts in the central nervous system to stimulate food intake via the growth hormone secretagogue receptor (GHSR) that is abundantly expressed in the ventral tegmental area (VTA). Not only does ghrelin modulate feeding behavior via a homeostatic mechanism, but numerous studies have identified ghrelin as a key regulator of reward-based hedonic feeding behaviors. Nutritional states influence ghrelin and GHSR expression as well as the behavioral sensitivity to reward-inducing stimuli. In the current study, we examined the role of ghrelin at the VTA level in food intake in two different nutritional states, satiety and hunger, by using a restricted feeding model. In this model, rats were conditioned to a daily 3-h (h) feeding session on standard chow for 10 days and a high-fat diet (HFD) was supplied either in the third hour after 2 h of chow diet intake, or at the beginning of a daily meal on the test day. We found that intra-VTA microinjection of 1, 2, and 4 μg of ghrelin, induced a dose-related increase of 1 h of reward-based feeding on HFD in sated rats, as well as a 24-h body weight gain. The overconsumption stimulated by ghrelin could be attenuated by 10 μg of direct infusion of the ghrelin receptor antagonist D-Lys3-GHRP-6 into the VTA. Moreover, our data showed that the injection of 1, 2, and 4 μg of ghrelin in the VTA, enhanced fasting-induced hyperphagia on HFD in a dose-related manner following a 21-h food restriction as well as a 24-h body weight gain. Conversely, hyperphagia on HFD that is potentiated by ghrelin could be blocked by pretreatment with a 10-μg D-Lys3-GHRP-6 intra-VTA microinjection. Collectively, these data demonstrate that ghrelin signaling at the VTA level mediates both reward-based eating and fasting-induced hyperphagia and provides a primary target for the control of the intake of rewarding food.

Key words: ghrelin, ventral tegmental area, high-fat diet, reward-based feeding, fasting-induced hyperphagia, nutritional state.

INTRODUCTION

It is well established that the gastric-derived peptide ghrelin plays an important role in the regulation of feeding and energy homeostasis (Kojima et al., 1999; Tschop et al., 2000; Zigman et al., 2005). The circulating levels of ghrelin increase before meals and during fasting, and this is consistent with its role in mealtime hunger and meal initiation (Cummings et al., 2001). Considerable evidence shows that ghrelin targets the hypothalamic and brain stem circuits involved in energy balance and elicits orexigenic effects by stimulating its unique specific receptor, the growth hormone secretagogue receptor type 1A (GHSR-1A) (Hewson and Dickson, 2000; Wren et al., 2001; Cowley et al., 2003; Faulconbridge et al., 2003). However, feeding behavior is not just driven by the need for nutrient repletion. Palatable high-fat food can also override the homeostatic signals and lead to overconsumption despite a state of satiety (Berthoud, 2004; Zheng and Berthoud, 2007), which is referred to as "reward-based feeding behavior" and thought to be a dominant factor in the current worldwide obesity epidemic.

Ghrelin interacts with GHSRs that are expressed in several reward-processing brain areas, such as the ventral tegmental area (VTA), the nucleus accumbens (NAcc), the prefrontal cortex, or the lateral hypo thalamus, implicating the role of ghrelin in the non-homeostatic, hedonic aspects of eating (Abizaid et al., 2006; Zigman et al., 2006). Research studies demonstrated that the direct injection of ghrelin into the VTA induced a robust chow intake and generated an interest in the possibility of ghrelin's action on reward feeding behaviors (Naleid et al., 2005; Abizaid et al., 2006). Moreover, intra-VTA administration of ghrelin increased the action potential frequency of VTA dopamine neurons (Abizaid et al., 2006) and induced the release of dopamine into the NAcc (Jerlhag et al., 2007). Reinforcing these findings, Abizaid et al. demonstrated that the blocking of VTA receptors with a selective GHSR antagonist in the VTA, blunts food intake induced by peripheral ghrelin

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Abbreviations: ANOVA, analysis of variance; ARC, arcuate nucleus; fMRI, functional magnetic resonance imaging; GHSR, growth hormone secretagogue receptor; GHSR-1A, growth hormone secretagogue receptor type 1A; HFD, high-fat diet; NAcc, nucleus accumbens; VTA, ventral tegmental area.

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and blunts rebound feeding following overnight fasting (Abizaid et al., 2006).

Given the key role of the VTA in reward behaviors together with ghrelin's established orexigenic effects, a wealth of studies on ghrelin's role in food reward behaviors were carried out. Administration of ghrelin in the VTA selectively increased the intake of high calorie palatable food but not the standard chow diet in a free choice paradigm (Egecioglu et al., 2010), although ghrelin action with the VTA could stimulate the intake of chow food when no other food is available (Naleid et al., 2005; Abizaid et al., 2006; Skibicka et al., 2013). Several studies identified the role of ghrelin in defining food preferences in the VTA. Ghrelin signaling enhances a preference for sweet and fatty foods (Shimbara et al., 2004; Disse et al., 2010). Likewise, the GHSR antagonist selectively decreased the intake of a 5% sucrose solution in a sucrose vs water two-bottle-choice drinking protocol (Landgren et al., 2011). In addition to enhancing the preference for palatable foods, ghrelin also increased the rewarding properties of a high-fat diet (HFD) and the motivation to obtain preferred foods (Perello et al., 2010; King et al., 2011; Skibicka et al., 2011, 2013). Ghrelin's effects on the rewarding aspect of food intake are also relevant in humans. The relevance was suggested by a functional magnetic resonance imaging (fMRI) analysis showing that ghrelin increases the neural response to food pictures in some reward-related brain centers, including the substance nigra and the VTA (Malik et al., 2008). This finding was further supported by another imaging study in humans showing that ghrelin can mimic the effects of fasting on the reward networks (Goldstone et al., 2014). Taken together, these findings suggest that the central ghrelin signaling in the VTA mediates food reward processing.

The nutritional states markedly influence the expression of ghrelin and GHSR as well as the behavioral sensitivity to the effect of reward-inducing stimuli. (Carr, 2002; Kim et al., 2003; Cummings, 2006). Anatomical and neurochemical correlates of altered behavioral sensitivity have been investigated. For instance, chronic mild food restriction increases the burst firing of dopamine neurons in mice (Branch et al., 2013), enhances the release of dopamine in the NAcc and the phasic dopamine signaling evoked during feeding compared with *ad libitum* feeding (Wilson et al., 1995; Cone et al., 2014). These findings imply that the orexigenic effect of ghrelin in the VTA may be influenced by the energy balance state. Thus in this study, we developed a restricted feeding model in which rats were subjected to a daily 3-h feeding schedule on a standard normal chow diet for 10 days and HFD was supplied either in the third hour after 2 h of chow diet intake or at the beginning of the daily meal on the test day. We investigated whether ghrelin infusion into the VTA would mediate (1) the reward-based feeding on HFD in calorically-sated rats; (2) the fasting-induced hyperphagia on HFD in food-deprived rats; and (3) the 24-h body weight gain. We further tested whether this effect would be attenuated by the pharmacological blockage of ghrelin signaling within the VTA to investigate whether the stimulation on food intake depends on the activation of GHS-R1A in the VTA.

EXPERIMENTAL PROCEDURES

Animals and surgery

Forty-two adult male Sprague–Dawley rats (provided by the Medical Experimental Animal Center of Xi'an Jiaotong University, Shaanxi Province, China) weighing between 250 and 300 g were housed individually in stainless-steel cages with free access to a standard chow diet and tap water. The rats were exposed to 12 h of light per day and the room temperature was maintained at 25 ± 1 °C. All animal procedures were approved by the Institutional Animal Care and Use Committee of Xi'an Jiaotong University. All efforts were made to reduce animal discomfort and the number of animals used.

Rats were anesthetized with chloral hydrate (300 mg/kg) that was injected intraperitoneally and were fitted with unilateral (VTA) 23-gauge stainless steel guide cannulas (Plastics One Inc., Roanoke, VA, USA). Stereotaxic coordinates taken from the rat brain atlas by Paxinos and Watson were as follows: VTA – 1.0 mm lateral, 5.3 mm posterior to Bregma, and 7.1 mm below the skull surface. The injector (30-gauge) extended 1 mm beyond the tip of the cannulas. Cannulas were fixed to the skull with the use of the dental acrylic resin and jeweler screws. A 30-gauge metal obturator filled the cannulas between tests. All the rats were injected with penicillin (20,000 units, ip) during the first three postoperative days to prevent infection, and the rats were allowed to recover for at least 7 days. During the 7-day recovery period, the animals were handled daily to habituate them to the injection procedure. All the experiments were conducted 4 h after the lights came on.

After all the experiments were complete, the rats were given an overdose of chloral hydrate and perfused transcardially with PBS, followed by 10% buffered formalin. The brains were removed, fixed, frozen-sectioned (40 μm) in a coronal plane, and stained with Cresyl Violet. The sites of the drug injections were identified according to Paxinos and Watson's atlas (Paxinos and Watson, 2007).

Drug microinjection

Ghrelin (Alexis Corporation., Lausen, CH, Switzerland) and the ghrelin receptor antagonist D-Lys3-GHRP-6 (Sigma–Aldrich Co. LLC, St. Louis, MO, USA) were dissolved in 0.9% sodium chloride immediately before the experiments. The drugs (0.5 μL each) were microinjected unilaterally into the VTA using 10 μL microsyringes (Hamilton Company, Reno, NV, USA) over a period of 60 s, with a 30-s delay before removing the injector. Each injector was attached to a microsyringe with a PE-20 tubing. After the injections, the obturators were replaced and the rats were placed back in their cages.