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INVESTIGATION OF A ROLE FOR GHRELIN SIGNALING IN BINGE-LIKE FEEDING IN MICE UNDER LIMITED ACCESS TO HIGH-FAT DIET

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Abstract—Binge eating is defined by the consumption of an excessive amount of food in a short time, reflecting a form of hedonic eating that is not necessarily motivated by caloric need. Foods consumed during a binge are also often high in fat and/or sugar. Ghrelin, signaling centrally via the growth-hormone secretagogue receptor (GHSR), stimulates growth hormone release and appetite. GHSR signaling also enhances the rewarding value of palatable foods and increases the motivation for such foods. As ghrelin interacts directly with dopaminergic reward circuitry, shown to be involved in binge eating, the current studies explored the role of GHSR signaling in a limited access model of binge eating in mice. In this model, mice received either intermittent (INT) or daily (DAILY) access to a nutritionally complete high-fat diet (HFD) for 2 h late in the light cycle, alongside 24-h *ad libitum* chow. In CD-1 mice, 2-h exposure to HFD generated substantial binge-like intake of HFD, as well as a binge-compensate pattern of 24-h daily intake. INT and daily groups did not differ in 2-h HFD consumption, while INT mice maintained stable intake of chow despite access to HFD. GHSR knock-out (KO) and wild-type (WT) mice both binged during HFD access, and exhibited the same binge-compensate pattern. INT GHSR KO mice did not binge as much as WT, while DAILY KO and WT were comparable. Overall, GHSR KO mice consumed fewer calories from HFD, regardless of access condition. GHSR KO mice also had reduced activation of the nucleus accumbens shell, but not core, following HFD consumption. These data support the ability of INT HFD in mice to induce a binge-compensate pattern of intake that emulates select components of binge eating in humans. There also appears to be a role for GHSR signaling in driving HFD consumption under these conditions, potentially via mediation of reward-related circuitry.

Key words: binge eating, intermittent access, high-fat diet, GHSR, ghrelin, nucleus accumbens.

INTRODUCTION

Binge eating is defined by the consumption of an excessive amount of food in a short time frame and is often associated with a sense of loss of control over the ability to cease eating once nutritional needs have been met (APA, 2013). Binge eating, therefore, reflects a form of hedonic eating that is not motivated by the necessity for calories or specific macronutrients, and is likely to be regulated in part by mesolimbic reward circuitry (Davis et al., 2009; for a review, see Bello and Hajnal, 2010). In support of this view, it is most often highly-palatable and energy-dense foods that are consumed during a binge episode (Kales, 1990; Elmore and DiCastro, 1991), and consumption of such foods are known to influence the short- and long-term plasticity of both homeostatic and brain reward circuitry (Bello et al., 2009; Johnson and Kenny, 2010). The precise mechanisms underlying the eating behavior seen in individuals that binge eat remain poorly understood. Identifying the factors that contribute to binge eating in both normal weight and obese binge eating disorder (BED) models, components of which may be dissociable, will aid in the understanding of the disorder and ideally improve treatment options for those that binge eat.

A number of animal models of binge-type eating have been developed (Corwin et al., 1998; Boggiano et al., 2007; Berner et al., 2008; Bello et al., 2009; Lardeux et al., 2013; Bake et al., 2014a). The limited access model, first demonstrated in rats by Corwin et al. (1998), is used to elicit binge-like consumption and alter patterns of fat intake that emulate select components of eating behaviors seen in humans with BED. This model is based on findings which show that restricting access to a substance can reliably enhance intake when that substance again becomes available (Wayner and Fraley, 1972; Pinel and Huang, 1976; Corrigall and Coen, 1989), and is thought to partially underlie the repeated failures of dieters who attempt to self-restrict intake of energy dense foods. In this model, rats placed under time restricted and sporadic access to fat demonstrate a binge-compensate pattern of feeding, such that they consume a greater number of calories on access days, and a reduced number of calories on non-access days relative to the control group who do not receive fat (Corwin et al., 1998). While this model does not accelerate the

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Abbreviations: BED, binge eating disorder; BSA, bovine serum albumin; DA, dopamine; GHSR, growth-hormone secretagogue receptor; HFD, high-fat diet; INT, intermittent; KO, knock-out; LHA, lateral hypothalamic area; NAcc, nucleus accumbens; SEM, standard error of the mean; TH, tyrosine-hydroxylase; VTA, ventral tegmental area; WT, wild type.

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development of obesity in all studies, it can alter patterns of intake and dietary composition, which may be reflected in changes in endocrine function and central nervous system activation.

Recent studies examining the impact of intermittent (INT) but regularly scheduled, and therefore predictable, access to high-fat diet (HFD) found that rats with INT access exhibit a number of metabolic consequences, such as impaired glucose tolerance, increased insulin levels and increased fat mass, relative to controls (Bake et al., 2014b). Interestingly, the lack of alterations in both orexigenic and anorexigenic peptide gene expression in the arcuate nucleus of the hypothalamus (ARC) prior to scheduled HFD or sucrose access in mice and rats, suggests that alternate mechanisms may be involved in the regulation of such binge-eating behavior (Bake et al., 2013). The excessive intake under such access conditions may be driven by reward systems that supplant the hypothalamic homeostatic control of feeding. Rats allowed INT scheduled access to a sweet-fat mixture not only consumed more food, but also exhibited higher terminal ghrelin levels as compared to rats with continuous access (Bello et al., 2009), suggesting that ghrelin signaling may play a role in increasing consumption of the palatable food when access is restricted. Elevated ghrelin levels have also been found in rats that developed binge-like eating when exposed to chow followed by a sweet-fat mixture subsequent to 2 h of daily food deprivation (Cottone et al., 2008).

Ghrelin, is a 28-amino acid peptide that is produced primarily by endocrine mucosal cells in the stomach (Kojima et al., 1999; Date et al., 2000) and its predominant functions include the stimulation of growth hormone release and the regulation of food intake and energy balance, both in the short- and long-term (Cummings, 2006). Ghrelin plays a role in meal initiation by peaking just prior to a meal in schedule fed humans and rodents (Cummings et al., 2001; Drazen et al., 2006). Elevated levels of ghrelin also result in a greater accumulation of body fat stores and a concomitant reduction in physical activity, leading to increased weight gain and reduced basal metabolic rate (Tschop et al., 2000). Ghrelin binds to the growth-hormone secretagogue receptor of the 1A subtype (GHSR; Howard et al., 1996), present at appreciable levels in widespread regions of the rat and mouse brain as detected by *in situ* hybridization (Guan et al., 1997; Zigman et al., 2006). Outside of ghrelin's actions on homeostatic nuclei in the ARC (for review see Horvath et al., 2001; Abizaid and Horvath, 2008), ghrelin exerts a substantial influence on reward circuitry (for review see: Abizaid and Horvath, 2008; Abizaid, 2009; Dickson et al., 2011; Skibicka and Dickson, 2011a, Perello and Dickson, 2015). Both systemic and direct ventral tegmental area (VTA) ghrelin administration enhances the release of dopamine (DA) in the nucleus accumbens (NAcc) (Abizaid et al., 2006; Jerlhag et al., 2006, 2007) and increases excitatory inputs onto VTA DA producing cells that co-localize with GHSR (Abizaid et al., 2006). Ghrelin administration also increases the rewarding value of highly palatable food (Egecioglu et al., 2010; Perello et al., 2010) as well as the motivation to work for such foods (Skibicka et al., 2011b, 2012; King et al., 2011). Given the ability of ghrelin to interact directly with dopaminergic reward circuitry known to be involved in binge eating, it could potentially be involved in the development of binge eating behaviors generated by INT access to highly palatable foods in mice.

To date, the role of ghrelin in binge eating in humans has been equivocal. An early study implicated the presence of the Leu72Met variant of the ghrelin gene in the development of BED in a small cohort of human subjects (Monteleone et al., 2007). Additional studies have reported that plasma ghrelin is reduced in obese-BED individuals (Geliebter et al., 2005; Monteleone et al., 2005) as well as in those with a higher frequency and severity of binge-purge behaviors (Troisi et al., 2005). Interestingly, the post-prandial suppression of ghrelin is also attenuated in obese-BED populations (Geliebter et al., 2005), which may reduce the satiating effect of a meal and may contribute to over-eating in the short term. It is currently unknown whether ghrelin signaling in normal-weight populations of humans or rodents is important for the development of binge eating on high-fat foods.

We initially set out to establish whether the limited access model utilized in rats (Corwin et al., 1998; Davis et al., 2007) would be reproducible in mice using a nutritionally complete HFD. In Experiment 1, we hypothesized that subjecting adult male mice to a limited access model of HFD, allowing access for 2 h per day 3 times a week, would elicit a binge/compensate pattern of caloric intake, relative to both mice fed a HFD for 2 h daily and chow-fed controls. Given ghrelin's central role in driving food intake and enhancing food reward, in our second experiment we hypothesized that mice lacking intact ghrelin receptors (*GHSR-KO*) would exhibit an attenuated binge-like pattern of eating typically demonstrated under this access schedule in wild-type (WT) rodents. We also examined differences between the mice in terms of neuronal activation in reward-related brain regions using c-Fos immunohistochemistry.

EXPERIMENTAL PROCEDURES

Animals

Experiment 1. Twenty adult male CD-1 mice (Charles River, St. Constant, QC) weighing 35–45 g were housed individually in a temperature and humidity controlled vivarium with a 12:12 light/dark schedule (Lights on: 07:00 to 19:00). All mice had *ad libitum* access to water and nutritionally complete standard laboratory chow (Teklad Global 14% Protein Rodent Maintenance Diet 2014, Harlan Laboratories, Madison, WI, USA; percent of kilocalories derived from fat: 13, protein: 20, carbohydrates: 67; 2.9 kcal/g) for the duration of the study. After one week of acclimatization to the vivarium environment, bodyweight and food intake were measured for 7 days to serve as a baseline. At the end of the baseline period, mice were given access to a nutritionally complete HFD overnight (Open Source