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Research report

Olfactory bulbectomy increases food intake and hypothalamic neuropeptide Y in obesity-prone but not obesity-resistant rats

Stefany D. Primeaux *, Maria J. Barnes, George A. Bray

Dietary Obesity Laboratory, Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, Louisiana 70808, USA

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Abstract

Obese individuals often suffer from depression. The olfactory bulbectomy (OBX) model is an animal model of depression that produces behavioral, physiological, and neurochemical alterations resembling clinical depression. The OBX model was employed to assess depression-related changes in food intake in obesity-prone, Osborne–Mendel (OM) rats and obesity-resistant, S5B/Pl rats. OBX increased food intake in OM rats beginning 7 days following surgery, however, OBX did not alter food intake in S5B/Pl rats at any time point. Fourteen days following surgery, OBX significantly increased locomotor activity (total lines crossed and rears) in the openfield test in OM and S5B/Pl rats. Fifteen days following surgery, prepro-neuropeptide Y (NPY) mRNA levels were significantly increased in the hypothalamus of bulbectomized OM rats and in the medial nucleus of the amygdala of bulbectomized OM and S5B/Pl rats. OBX decreased NPY Y2 receptor mRNA levels in the hypothalamus and medial nucleus of the amygdala in OM rats, while increasing NPY Y2 receptor mRNA levels in the medial nucleus of the amygdala of S5B/Pl rats. These data indicate that though both obesity-prone and obesity-resistant strains were susceptible to the locomotor effects of OBX, food intake and hypothalamic prepro-NPY mRNA were only increased in OM rats. Therefore, strain specific alterations in hypothalamic NPY may account for increased food intake in the obesity-prone rats following OBX, and suggests a potential mechanism to explain the comorbidity of obesity and depression.

Keywords: Olfactory bulbectomy; Depression; Food intake; Neuropeptide Y; Obesity-prone; Hypothalamus; Amygdala

1. Introduction

Obesity is an increasingly prevalent medical condition associated with a variety of health conditions and mood disorders, including depression. Higher body mass indexes (BMIs) have been linked to depressive symptoms in adults and adolescents. The DSM IV (1994) reports symptoms of major depression as changes in appetite or weight, sleep, psychomotor activity, irritability, anxiety, loss of interest in previously pleasurable activities, impaired ability to concentrate and depressed mood. To be diagnosed with major depressive disorder, an individual must have either depressed mood or loss of interest or pleasure and four of the other symptoms. According to the DSM IV, major depressive disorder with atypical symptoms is characterized by increased food intake and body weight. Twins with atypical depression are more likely to be obese (BMI > 28.6) than those with other forms of depression [18,40,54,63].

The olfactory bulbectomy (OBX) animal model of depression produces a syndrome characterized by behavioral, physiological and neurochemical changes that resemble clinical depression. This syndrome is behaviorally characterized by increased locomotor activity in a novel openfield [17,36,41,43,61], deficits in aversively motivated behaviors [29,41–43] and deficits in appetitively motivated behaviors [4,30,31,43]. Alterations in feeding patterns and circadian rhythms have been seen in bulbectomized Sprague–Dawley rats [27,30,31,39]. Though total food intake was not altered, these rats eat smaller, but more frequent meals [30,31]. Kelly et al. [16] reported a significant increase in food intake at a single time point (11 days) following OBX.

There are many neurochemical similarities between obesity and depression [2,7,28,60]. Specifically, the 36 amino acids neuropeptide, neuropeptide Y (NPY), has been implicated in both disorders [8,9,23,33,42,58]. Animal models indicate that the orexigenic effects of NPY are mediated through the hypothala-

* Corresponding author. Tel.: +1 225 763 2736; fax: +1 225 763 2525.
E-mail address: PrimeaSD@pbrc.edu (S.D. Primeaux).

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mus, in particular by NPY neurons that project from the arcuate nucleus to the paraventricular nucleus [1,50,51]. Both intracerebroventricular and intra-paraventricular nucleus administration of NPY increases food intake [13,32,56,58] with the macronutrient specificity of the response being somewhat dependent on the rat's original preference for carbohydrate or fat [6,55,57,62]. Chronic central administration of NPY and NPY Y1 receptor agonists increases body weight and adiposity and produces significant hyperphagia [9].

In addition to it's important role in ingestive behaviors, the role of NPY has been investigated in a number of animal models of anxiety and depression [3,10,12,19,52,59,66]. Though no studies have fully investigated NPY alterations in the hypothalamus of bulbectomized rats, increased NPY mRNA levels and peptide levels in the piriform cortex and medial nucleus of the amygdala have been found in the OBX animal model of depression [10,42,48]. The medial nucleus of the amygdala is an important brain region involved in both ingestive behaviors and emotional behaviors, such as anxiety and depression [5,26,38,64]. Lesions of the medial nucleus of the amygdala lead to increased food intake and body weight [21,22].

The Osborne–Mendel (OM) rat and the S5B/Pl rat are animal models used to assess sensitivity toward developing obesity. When compared to obesity-resistant, S5B/Pl rats, obesity-prone OM rats become obese when given access to a high fat diet (55% of energy from fat) and consume more calories from fat than carbohydrates when given a choice [34]. In addition to the OM rat's propensity toward obesity, these animals have increased hypothalamic prepro-NPY, NPY Y1 and Y2 receptor mRNA when compared to obesity-resistant S5B/Pl rats [20,49,53]. The goal of the current experiment was to examine the effects of OBX on body weight, food intake, locomotor activity, and NPY gene expression in obesity-prone and obesity-resistant rats. We hypothesized that OBX would increase locomotor activity in both strains of rats, but would selectively increase food intake in obesity-prone OM rats. Increased food intake in bulbectomized OM rats would suggest that these rats are representative of individuals with major depression with atypical symptoms, which includes increased food intake. Similar to what was previously reported in Sprague–Dawley rats [27,30,31,37], we do not expect to find OBX-induced increases in food intake in S5B/Pl rats. NPY and NPY receptor gene expression was assessed in the hypothalamus and medial nucleus of the amygdala following OBX by real-time PCR. We predict that OBX will increase prepro-NPY mRNA levels in the medial nucleus of the amygdala in both strains, though we expect differential expression of NPY and NPY receptor subtypes in the hypothalamus.

2. Materials and methods

2.1. Subjects

Eight-week-old male OM and S5B/Pl rats, bred in the Pennington Biomedical Research Center vivarium, were used in this experiment. Rats were individually housed (beginning at 7 weeks of age) on a 12/12 LD cycle (lights on at 07:00) with food/water available *ad libitum*. Rats were fed a standard laboratory chow diet (Rodent Diet 5001, LabDiet; 28/12/60: % calories from protein/fat/carbohydrates). All procedures were approved by the Pennington Biomedical Research Center Institutional Animal Care and Use Committee and followed the *Principles for Care and Use of Laboratory Animals*. This facility is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC).

2.2. Olfactory bulbectomy

OBX surgery was performed as previously described [10,41]. Briefly, rats were anesthetized with sodium pentobarbital (Nembutal, Abbot Laboratories, 55 mg/kg, i.p.) and the scalp was shaved. Following a midline incision, two 2 mm diameter burr holes were drilled 6 mm rostral to bregma and 1 mm to the right and left of the midline. The olfactory bulbs were aspirated in the bulbectomized rats with a 2 mm diameter plastic pipette tip and the cavity was filled with gelfoam (Upjohn) to control bleeding. Special care was taken to avoid damaging the frontal cortex. Carprofen (Rimadyl, Pfizer, 1 mg/kg, s.c.) was administered immediately following surgery to control pain. Sham-operated controls were treated identically except the olfactory bulbs were not aspirated. Immediate post-operative care consisted of fluid replacement (.9% saline, i.p.) and thermoregulatory measures. Rats were monitored daily following surgery for any signs of distress.

2.3. Food intake and body weight

Twenty-four hours food intake was measured for 2 days prior to OBX surgery to determine the average 24 h baseline food intake for each strain. Following OBX, 24 h food intake was measured daily beginning 6 days following surgery and continuing through day 15. Body weight was also measured daily following surgery.

2.4. Openfield testing

Fourteen days following surgery, all animals were tested in the openfield test. The openfield apparatus is a four-sided 50 cm × 50 cm × 50 cm black Plexiglas chamber, with a stainless steel floor. The floor of the openfield was divided into nine equal squares (16.67 cm). Rats were placed into the middle of the openfield apparatus, initially. Each rat was given 5 min to explore the openfield, during which time they were videotaped. The number of rears and the number of lines crossed in the openfield were used as measures of spontaneous locomotor activity and manually assessed by the experimenter. The experimenter was blind to the treatment condition during testing and during the measurement of activity. The openfield was cleaned with a 20% chlorine bleach solution between each animal in an attempt to eliminate odors.

2.5. Real-time polymerase chain reaction (PCR)

One day following openfield testing, S5B/Pl rats and OM rats were killed by rapid decapitation. Dissected brains were frozen on dry ice, and stored at −80 °C until processing. RNA was isolated from bilateral punches of the hypothalamus and medial nucleus of the amygdala using Tri-Reagent (Molecular Research Ctr, Cincinnati, OH, USA) and RNeasy Minikit procedures (Qiagen, Valencia, CA, USA) and based on previous experiments by Primeaux et al. [44]. Briefly, thawed tissue was homogenized in Tri-Reagent using a motorized tissue homogenizer, chloroform (200 μl) was added to the lysate, and the mixture was centrifuged (12,000 × *g*) in phase lock tubes to separate RNA. Ethanol (600 μl) was added to the upper aqueous phase, which was filtered by centrifugation (8000 × *g*). Following three washes, the samples were subjected to an elution step using RNAase-free water. Reverse transcriptase (RT) was conducted using M-MLV procedures (Promega, Madison, WI, USA). For RT, 2 μg of RNA from each sample was added to random primers (Promega) and incubated in a thermal cycler (PTC-100, MJ Research Inc., Watertown, MA, USA) for 5 min at 70 °C. Tubes were removed, placed on ice and a mixture of 5× M-MLV (Moloney Murine Leukemia Virus), 10 mM dNTP (solution containing sodium salts of dATP, dCTP, dGTP and dTTP) and RT buffer (250 mM Tris–HCl (pH 8.3 at 25 °C), 375 mM KCl, 15 mM $MgCl_2$, 50 mM DTT) was added and tubes were returned to the thermal cycler for 60 min at 37 °C and then 15 min at 70 °C. Primers were designed using Primer Express (Applied Biosystems, Foster City, CA, USA). The