Brief Communication

MOLECULAR METABOLISM

The reward value of sucrose in leptin-deficient obese mice

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ABSTRACT

Leptin-deficient patients report higher "liking" ratings for food, and leptin replacement therapy normalizes these ratings even before weight loss is achieved. Since animals cannot report their ratings, we studied the relationship between leptin and food reward in leptin-deficient *ob*/*ob* mice using a optogenetic assay that quantifies the reward value of sucrose. In this assay, mice chose between one sipper dispensing the artificial sweetener sucralose coupled to optogenetic activation of dopaminergic (DA) neurons, and another sipper dispensing sucrose. We found that the reward value of sucrose was high under a state of leptin deficiency, as well as at a dose of leptin that does not suppress food intake (12.5 ng/h). Treatment with higher doses of leptin decreased the reward value of sucrose before weight loss was achieved (100 ng/h), as seen in leptin-deficient patients. These results phenocopy in mice the behavior of leptin-deficient patients.

Keywords Sucrose; Food preference; Reward value; Optogenetics; Leptin; Obesity

1. INTRODUCTION

Leptin is an adipose tissue hormone that functions as an afferent signal in a negative feedback loop that maintains relative constancy of adipose tissue mass [3]. Leptin functions as a homeostatic signal to control food intake, and recent studies have shown that leptin reduces food intake in part by diminishing the reward value of sucrose [1,2]. In humans, the reward value of food is assessed using a subjective rating scale for "liking". Leptin-deficient patients report higher "liking" ratings for food, and leptin replacement therapy normalizes these ratings even before weight loss is achieved [1]. Assays of "liking" in rodents are limited by the fact that animals cannot verbally report their ratings. In order to assay "liking" in mice, we recently developed a two-bottle choice assay that measures preference for sucrose versus a reference stimulus in which ingestion of sucralose induces optogenetic stimulation of dopaminergic (DA) neurons [2,4,5]. In this assay, changes in preference relative to this optogenetic reference stimulus reflect changes in the reward value of the nutrient in the second bottle, in this case sucrose [2]. Using this assay, we previously accessed in wild-type mice the reward value of sucrose under different metabolic states – food restriction leading to weight loss increases the reward value of sucrose, and this increase is reversed by leptin. However, these results involved weight loss, and hence do not dissociate the hormonal effect from an effect of the body mass. In this report, we studied the effects of leptin treatment of *ob* mice on the reward value of sucrose before significant weight loss is achieved. Our results phenocopy the behavior of leptin-deficient humans, in which leptin treatment normalized the reward value of food even before weight loss.

2. MATERIALS AND METHODS

2.1. Animals

Dat-cre transgenic mice were obtained from the Laboratory of Nils-Goran Larsson, Karolinska Institutet [6]. Heterozygous *ob*/+ mice were obtained from Jackson Laboratories. Animals of either sex were kept in C57BL/7 background. Unless specified otherwise, mice were single housed and maintained under an inverted 12-h light/dark cycle, so that behavioral tests could be performed during the rodent active phase (dark). Standard chow and water were provided *ad libitum*, except during acclimatization to the behavioral chambers and laser training (see Section 2.3). Experimental protocols were approved by The Rockefeller University IACUCs and met the guidelines of the national Institutes of Health guide for the Care and Use of Laboratory Animals.

2.2. Virus preparation

To construct Cre-inducible recombinant AAV vectors, the DNA cassette carrying two pairs of incompatible lox sites (loxP and lox2722) was synthesized and the ChR2-mCherry transgene was inserted between the loxP and lox2722 sites in the reverse orientation [4]. The resulting

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double floxed reverse ChR2-mCherry cassette was cloned into a modified version of the pAAV2-MCS vector carrying the EF-1α promoter and the Woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) to enhance expression [4]. The recombinant AAV vectors were serotyped with AAV5 coat proteins and packaged by the viral vector core facility at the University of North Carolina. The final viral concentration was 2×10^{12} genome copies/mL [4].

2.3. Surgeries

Surgical procedures were adapted from [2]. Virus or vehicle was injected via a pressure injector through pulled glass pipettes, which were positioned to the ventral tegmental area (VTA) on coordinates AP = −3.5 mm ML = ±0.5 mm, DV = 4.8 mm (Paxinos) with a stereotaxic frame. The fiber optic ferrule (Thorlabs) was chronically implanted with a stereotaxic frame, being secured with dental cement (Lang), and aimed at the aforementioned coordinates. Animals were allowed to recover from surgery for one week prior to any behavioral assays.

2.4. Behavioral and optogenetic setup

The lick-induced optogenetic stimulation has been described in our previous publication [2]. Briefly, MedAssociates chambers were equipped with two contact lickometers and a laser source (solid state Crystal laser, 473-nm wavelength) controlled by MedPC via a TTL impulse to be triggered upon lick detection. The laser turns on every five consecutive licks on the same sipper, being ON for one second and OFF for the following second. Animals were water deprived for 16–23 h, and acclimated to the chambers until side preference for either sipper was even, which required about one week. During the acclimation period mice were water deprived and were given water through the sippers inside the chamber for half an hour. Two-bottle preference was calculated as the ratio: preference for sipper1 = number of licks on sipper1/(number of licks on sipper1 + number of licks on sipper2) and expressed as percentage values, with 50% representing the indifference ratio. Behavioral data were analyzed with Excel, and expressed as mean ± SEM. Significance tests comparing groups were *t*-tests, and, when appropriate, followed by Bonferroni corrections for multiple comparisons. After recovering from surgery animals were given water inside the chamber for half an hour, for four consecutive days. During day 1 mice were given water through one of the sippers, with the laser source being triggered upon licking as mentioned above (Laser Sipper = LS). Laser/sipper coupling was balanced. On day 2 mice were given water only through the other sipper, in the absence of laser activation (Control Sipper = CS). Day 3 and day 4 were, respectively, repetitions of days 1 and day 2. On the fifth day animals were given LS and CS simultaneously for 10 min. Once a clear bias towards LS was established (above 55%), animals were subjected to experiments testing the effects of different doses of leptin on the reward value of sucrose (Figures 3–5). Concentrations of sucrose and sucralose were set as published elsewhere and were, respectively, 140 mM and 0.5 mM [2].

2.5. Immunohistochemistry

Immunohistochemistry was performed following the protocols as published elsewhere [2], using a rabbit anti-TH (Pel-Freez, 1:1000) antibody and appropriate host secondary antibodies.

2.6. Leptin treatment

Recombinant mouse leptin was obtained from Amylin Pharmaceuticals (San Diego, CA) and administered through subcutaneous osmotic pumps (Alzet; Palo Alto, CA) filled with the indicated concentrations of leptin and incubated overnight at 37 °C in sterile 0.9% NaCl, and implanted subcutaneously in 10- to 14-week-old mice. Posology is described in Figure 2.

3. RESULTS

3.1. Optogenetic targeting of dopaminergic neurons in *ob/ob* mice

We directed the expression of Channelrhodopsin-2 (ChR2) to DA neurons of the midbrain of genetically *ob*ese *ob/ob* mice as follows (Figure 1): Dat-cre

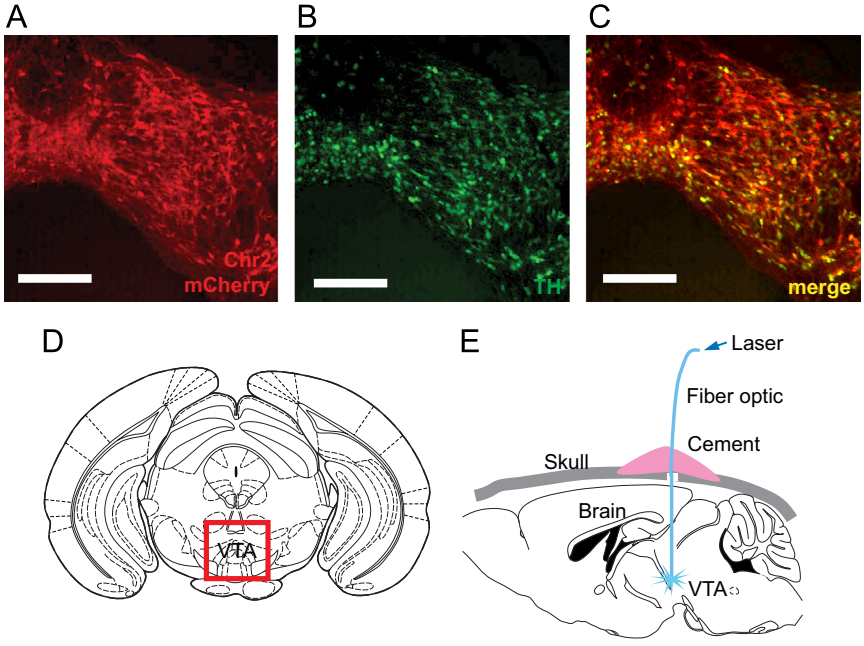

Figure 1: Tissue-specific expression of channelrhodopsin in DA neurons of *ob/ob* mice and cranial fiber optic implant. (a–c) AAV-DIO–ChR2-mCherry injection into the VTA of *ob/ob*;Dat-cre mice led to ChR2-mCherry expression in neurons colocalizing with tyrosine hydroxylase (TH), a marker for DA neurons (statistics imbedded in text, scale bars represent 100 μm). (d and e) Optical fibers were implanted above the VTA (red inset box in (d)) of *ob/ob*;Dat-cre mice.

