Trk B Signaling in Dopamine 1 Receptor Neurons Regulates Food Intake and Body Weight

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Objective: Loss of BDNF-TrkB signaling results in obesity in both humans and mice; however, the neural circuit that mediates this effect is unknown. The role of TrkB signaling in dopamine-1 receptor expressing neurons in body weight regulation was tested.

Design and Methods: Mice with a floxed allele of the TrkB gene were paired with mice expressing Cre-recombinase under control of the D1 promoter to conditionally knock out expression of TrkB receptors from D1-neurons.

Results: Deletion of TrkB receptors from D1 neurons results in obesity in chow fed mice due to increased feed efficiency. In contrast, loss of TrkB signaling in D1 neurons induced hyperphagia and hyperglycemia in mice maintained on high fat diet.

Conclusions: These findings indicate TrkB signaling in D1 neurons regulates body weight by distinct mechanisms for chow and high fat diet and may be important for defending the body against the development of obesity and obesity-related disorders.

Introduction

Brain-derived neurotrophic factor (BDNF) is a highly conserved neurotrophin that signals through the tropomyosin-related kinase B (TrkB) receptor (1). In addition to roles in neuronal development and synaptic plasticity, BDNF-TrkB signaling is appreciated as an important regulator of body weight. Mice heterozygous for BDNF have marked obesity that can be reversed by intraventricular infusion of BDNF (2). Mice with genetically reduced TrkB expression (~25% of normal) are hyperphagic and gain excessive weight on a high fat diet (3). Furthermore, genetic disruption of BDNF-TrkB signaling causes obesity in human subjects, thus confirming the importance of this pathway in human body weight regulation (4,5).

While BDNF signaling has been associated with control of feeding and body weight (1), the specific sites of TrkB action that mediate this effect are unknown. TrkB is broadly expressed in the brain, including most areas of the hypothalamus as well as the mesolimbic reward pathway, a major regulator of reward processing (1). During studies examining the role of TrkB signaling in the action of cocaine (6), it was noted that loss of TrkB in dopamine-1 receptor (D1) neurons resulted in weight gain, indicating that D1 neurons may be an important site of TrkB action on body weight. D1 neurons are expressed in brain regions known to regulate food intake, including the ventral striatum, and several hypothalamic nuclei including the paraventricular and suprachiasmatic nuclei (7). Therefore, we conducted a rigorous analysis on the role of D1 neurons in BDNF-TrkB-related body weight effects by crossing mice with a loxp-flanked allele of the TrkB receptor (8) to a mouse line expressing Cre-recombinase under control of the D1-promotor (D1-Cre).

Methods

Animals

Mice were housed in the University of Texas Southwestern Medical Center (UTSW) vivarium in a temperature-controlled environment (lights on: 06:00-18:00) with ad lib access to water and standard chow (SC: 4% fat diet #7001, Harlan-Teklad, Madison, WI). All animal procedures were performed in accordance with UTSW Institutional Animal Care and Use Committee guidelines. All mice used in this study were handled in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the US National Institutes of Health, and the specific protocols were approved by the Institutional Animal Care and Use Committee.
Results

Wild-type (non-loxP-flanked TrkB with or without D1-Cre), floxed-TrkB without D1-Cre (fTrkB) and floxed-TrkB with D1-Cre (fTrkB\(^{D1-Cre}\)) littermate male mice were individually housed and monitored for food intake and body weight for 8 weeks on both chow and a 42% high fat diet (HFD). On regular chow, while no difference in body weight was noted between wild-type and fTrkB mice, fTrkB\(^{D1-Cre}\) mice gained over 6 g more in body weight over the course of the 8-week experiment (Figure 1a). Analysis of cumulative food intake demonstrated no difference between fTrkB and fTrkB\(^{D1-Cre}\) groups (Figure 1b). A small, but statistically significant, increase in chow consumed was observed between both fTrkB and fTrkB\(^{D1-Cre}\) groups compared to wild-type mice, suggesting that creation of the floxed allele affects neurophysiology to promote a small increase in food intake without altering body weight gain.

Because BDNF-TrkB signaling has previously been implicated in the consumption of palatable foods, we next analyzed the effect of HFD on body weight in our three groups. We did not observe a difference in body weight between wild-type and fTrkB groups, but again fTrkB\(^{D1-Cre}\) mice gained significantly more than the other two groups (Figure 1d). Analysis of cumulative food intake demonstrated no significant difference in HFD intake between the wild-type and fTrkB groups, but a large increase in cumulative HFD consumption in fTrkB\(^{D1-Cre}\) mice (Figure 1e).

While body weight homeostasis was affected by loss of TrkB signaling in D1-neurons, it was not clear if the weight gain was a result of increased calorie intake or reduced energy expenditure. To begin to answer this question, we calculated feed efficiency (body weight gain/cumulative calorie intake) for all groups of mice during the 8-week period on both diets, taking into account the above food intake data. Compared to wild-type and fTrkB groups, fTrkB\(^{D1-Cre}\) mice displayed a significant increase in feed efficiency on chow (Figure 1c). Importantly, no difference in locomotor activity was noted between fTrkB and fTrkB\(^{D1-Cre}\) groups (2152 ± 124 vs. 2581 ± 252, \(P > 0.05\)), suggesting that reduced energy expenditure, and not a change in locomotor activity, is the primary cause of weight gain. In contrast, no difference in feed efficiency was noted between the three groups on HFD (Figure 1f), indicating that increased calorie consumption was the primary cause of the obesity observed in fTrkB\(^{D1-Cre}\) mice fed HFD.

Obesity is associated with increased risk for developing several metabolic disorders including type II diabetes and metabolic syndrome. Therefore at the end of the study, we performed a comprehensive metabolic profile on all three groups to determine if loss of TrkB signaling in D1R neurons predisposed to these metabolic disorders. Despite a significant weight gain in chow-fed fTrkB\(^{D1-Cre}\) mice (Figure 1a), no effect of genotype was observed on glucose or insulin levels in chow fed mice (Figure 2a and 2b). In contrast, loss of TrkB signaling in D1 neurons induced significant elevations in both fasting glucose and fasting insulin levels in fTrkB\(^{D1-Cre}\) mice (Figure 2c and 2d) suggesting insulin resistance. No significant effect of genotype was observed on fasting levels of triglycerides, total cholesterol, or high-density lipoprotein cholesterol (data not shown). Therefore, TrkB signaling in D1 neurons defends against the development of HFD-induced weight gain and associated conditions such as insulin resistance.
To our knowledge, this is the first evidence for a TrkB signaling role in feed efficiency and also identifies D1-neurons as a critical site of TrkB action on body weight. Furthermore, our data indicate that TrkB signaling in D1-neurons differentially regulates body weight depending on the diet, suggesting that BDNF-TrkB signaling may integrate dietary information differentially into coordinated metabolic responses that defend against obesity and obesity-related disorders such as insulin resistance. Such a possibility is in line previous reports that BDNF expression is differentially regulated by macronutrients. One report found that glucose infusion causes a rapid increase in BDNF mRNA in the ventromedial hypothalamus (8) while consumption of HFD reduces BDNF mRNA in the ventral tegmental area after 60 min (10).

Still unknown is the site of BDNF production that regulates TrkB signaling on D1-neurons. Dopaminergic neurons originating from the VTA are one likely candidate. As noted above, BDNF levels within the VTA are decreased after consumption of HFD and selective reduction of BDNF in the VTA increases consumption of HFD (10). The observation that HFD reduces BDNF mRNA levels in the ventral tegmental area is consistent with our findings that complete loss of TrkB signaling in D1-neurons leads to a dramatic increase of the highly palatable HFD. It has previously been hypothesized that loss of BDNF in the VTA increases caloric intake and body weight (12). The observation that loss of TrkB signaling in D1-neurons increases caloric intake and body weight is consistent with this hypothesis and suggests a role for TrkB signaling in the regulation of caloric intake and body weight.

**Discussion**

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of BDNF could affect reward processing in the ventral striatum leading to a “reward-deficiency syndrome” that promotes compensatory overeating (reviewed in Ref. 1). Indeed, deletion of TrkB from D1-neurons increases the conditioned place preference for cocaine (6), indicating that BDNF-TrkB signaling in D1 neurons does affect reward sensitivity. These observations suggest a model in which calorically dense foods, like HFD, promote overconsumption by reducing expression of BDNF within dopaminergic neurons and subsequent TrkB signaling in D1-neurons. Another possible source is the hypothalamus. Central infusion of BDNF induces neuronal activity in several hypothalamic nuclei and reduction of BDNF expression in the basomedial hypothalamus increases food intake and body weight (8). Finally, it is important to identify the intracellular signaling pathways that mediate the effect of TrkB signaling on body weight in D1-neurons. Research in the fields of drug addiction and stress implicates multiple consequences of TrkB signaling in D1-neurons on neuroplasticity, including alterations in CREB activity, ERK signaling and AMPA receptor trafficking (11-13). These new insights will help determine the role of neural adaptations in body weight regulation and protection from obesity and related disorders.

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