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MOLECULAR METABOLISM

Loss of neurotensin receptor-1 disrupts the control of the mesolimbic dopamine system by leptin and promotes hedonic feeding and obesity*

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ABSTRACT

Neurons of the lateral hypothalamic area (LHA) control motivated behaviors such as feeding and ambulatory activity, in part by modulating mesolimbic dopamine (DA) circuits. The hormone, leptin, acts via the long form of the leptin receptor (LepRb) in the brain to signal the repletion of body energy stores, thereby decreasing feeding and promoting activity. LHA LepRb neurons, most of which contain neurotensin (Nts; LepRb^{Nts} neurons) link leptin action to the control of mesolimbic DA function and energy balance. To understand potential roles for Nts in these processes, we examined mice null for Nts receptor 1 (NtsR1KO). While NtsR1KO mice consume less food than controls on a chow diet, they eat more and become obese when fed a high-fat, high-sucrose palatable diet; NtsR1KO mice also exhibit augmented sucrose preference, consistent with increased hedonic feeding in these animals. We thus sought to understand potential roles for NtsR1 in the control of the mesolimbic DA system and LHA leptin action. LHA Nts cells project to DA-containing midbrain areas, including the ventral tegmental area (VTA) and the substantia nigra (SN), where many DA neurons express NtsR1. Furthermore, in contrast to wild-type mice, intra-LHA leptin treatment increased feeding and decreased VTA *Th* expression in NtsR1KO mice, consistent with a role for NtsR1 signaling from LHA LepRb neurons in the suppression of food intake and control of mesolimbic DA function. Additionally, these data suggest that other leptin-regulated LHA neurotransmitters normally oppose aspects of Nts action to promote balanced responses to leptin.

Keywords Neurotensin; Obesity; Orexin; Dopamine

1. INTRODUCTION

The lateral hypothalamic area (LHA) receives and integrates signals, including metabolic stimuli, to modulate motivated behaviors (such as feeding, drinking and locomotor activity) as appropriate for current environmental conditions [1]. Part of this function is mediated by the projection of some LHA neurons to midbrain regions (the ventral tegmental area (VTA) and substantia nigra (SN)), which contain dopamine (DA) neurons [2] that regulate the incentive salience of food and locomotor activity, among other things [3].

The LHA contains several groups of neurons that contribute to energy balance, including glutamatergic orexin/hypocrectin (OX)-containing cells that are activated by signals of energy deficit, and which promote food-seeking and increased vigilance [4,5]. Other LHA neurons containing MCH promote feeding as well [6]. The LHA also contains a distinct population of neurons that express the neuropeptide neurotensin (Nts) [7]. Approximately 30% of LHA Nts neurons co-express the inhibitory neurotransmitter GABA and the leptin receptor (LepRb) and are referred to as LepRb^{Nts} neurons; leptin action via these LepRb^{Nts} neurons is crucial for the control of DA signaling, locomotor activity, and energy balance [8,9]. Some other LHA Nts neurons (non LepRb^{Nts}) may be glutamatergic, since activation of LHA Nts neurons promotes NMDA-mediated EPSCs in DA neurons of the VTA [10]. While the contributions of OX and MCH neuropeptide signaling in energy balance and motivated behaviors are beginning to be understood, the role of Nts signaling via LHA Nts neurons has remained unclear.

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Abbreviations: LHA, lateral hypothalamic area; DA, dopamine; LepRb, long form of the leptin receptor; Nts, neurotensin; NtsR1, neurotensin receptor-1; NtsR2, neurotensin receptor-2; NtsR1KO, neurotensin receptor-1 knock out; VTA, ventral tegmental area; SN, substantia nigra; TH, tyrosine hydroxylase; NAc, nucleus accumbens; OX, Orexin/hypocretin; MCH, melanin concentrating hormone; pSTAT3, phosphorylation of signal transducer and activator of transcription 3; PD, palatable diet

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Acute Nts administration activates midbrain DA neurons [11–13], promotes DA release to the NAc [12,14,15] and limits feeding [16–18]. Central Nts also site-specifically regulates locomotor activity: NAc Nts treatment attenuates locomotor activity, including blunting the locomotor response to psychostimulants [19–21] but Nts in the VTA promotes spontaneous locomotor activity [22–24]. These reports suggest that endogenous Nts released into the VTA might regulate energy balance (e.g. limit feeding and promote locomotor activity) by regulating midbrain DA signaling. Given that LHA Nts neurons project to the midbrain [9] we reasoned that these neurons might contribute to Nts-mediated regulation of VTA DA signaling and feeding. Additionally, we hypothesized that the population of $LepRb^{Nts}$ neurons within the LHA might link leptin signaling to the modulation of midbrain-regulated motivated behaviors that are relevant to energy balance [18,25,26]. Most central actions of Nts are mediated via neurotensin receptor-1 (NtsR1) and neurotensin-receptor-2 (NtsR2), which are 7-transmembrane G-protein coupled receptors expressed broadly throughout the brain [27]. NtsR2 mainly modulates analgesia [28–30]. NtsR1 signaling is required for VTA-stimulated locomotor activity [21] and regulation of feeding, including the suppression of food intake by leptin [16,17]. These findings suggest that NtsR1 might play a role in the control of motivated behaviors by LHA Nts neurons and leptin action. We therefore employed NtsR1-null (NtsR1KO) mice to define roles for NtsR1 signaling in the control of hedonic feeding, as well as the control of the mesolimbic DA system by LHA leptin action. Our data reveal an anatomic circuit via which LHA Nts neurons, including $LepRb^{Nts}$ neurons, modulate midbrain DA neurons to regulate energy balance, and disruption of Nts/NtsR1 signaling via this circuit may promote hedonic intake and the development of obesity.

2. MATERIALS AND METHODS

2.1. Materials

Recombinant mouse leptin was the generous gift of Amylin Pharmaceuticals (La Jolla, CA).

2.2. Animals

Wild-type (WT) and *NtsR1KO* (*Ntsr1*^{tm1Dgen}; Stock # 005826) mice on the C57BL/6 background were purchased from Jackson Laboratories (Bar Harbor, ME) and bred in our colony to produce study animals. *Nts*^{cre} mice (*Nts*$^{tm1(cre)Mgmj}$/*J*; Jackson stock # 017525) used for tract tracing studies were generated as described previously [9]. *NtsR1*^{cre} animals (*Tg*(*Ntsr1-cre*)*GN209Gsat*/*Mmucd*, Stock # 030780-UCD) were purchased from the Mutant Mouse Regional Resource Center (Davis, CA) and interbred with ROSA26-tdTomato reporter mice (*Gt*(*ROSA*)*26Sor*$^{tm9(CAG-tdTomato)Hze}$/*J*; Jackson Stock # 006148) to facilitate expression of a red fluorescent protein variant (tdTomato) within cre-expressing neurons. All animals were housed and bred within our colony according to guidelines approved by the University of Michigan Committee on the Care and Use of Animals and the Michigan State University Institutional Animal Care and Use Committee. Mice were given ad libitum access to food and water unless otherwise noted in experimental methods. Male mice were used for all studies.

2.3. Metabolic profiling of NtsR1KO mice and controls

WT and NtsR1KO mice were weaned and single housed at 3 weeks of age. Mice were subsequently maintained for 12 weeks on either a standard rodent chow or a palatable diet that was high in fat and sucrose (PD, Research Diets #12451: 45 kCal% fat, 35 kCal% carbohydrate and 20 kCal protein). Animals were then analyzed via the Comprehensive Laboratory Monitoring System (CLAMS, Columbus Instruments), an integrated open-circuit calorimeter equipped with an optical beam activity-monitoring device. Mice were weighed before being individually placed into the sealed chambers (7.9″ × 4″ × 5″) with free access to food and water. The study was carried out in an experimentation room set at 20–23 °C with 12–12 h (6:00PM–6:00AM) dark–light cycles. The measurements were carried out continuously for 48–72 h, and data were used from the last 24 h, at which point animals are sufficiently acclimated to the chambers. The amount of food of each animal was monitored through a precision balance attached below the chamber. The system was routinely calibrated each time before the experiment using a standard gas (20.5% O_2 and 0.5% CO_2 in N_2). VO_2 and VCO_2 in each chamber were sampled sequentially for 5 s in a 10 min interval and the motor activity was recorded every second in X and Z dimensions. The air-flow rate through the chambers was adjusted to keep the oxygen differential around 0.3% at resting conditions. After CLAMS analysis, animals were returned to their home cages and allowed to recover for 1 week prior to euthanasia and dissection of tissues, which occurred after mice had been on chow or PD for 16–17 weeks.

2.4. Sucrose preference testing

To measure sensitivity to reward we analyzed WT and NtsR1KO animals (14–16 weeks of age) via a two-bottle sucrose preference testing paradigm using a Volumetric Drinking Monitor (Columbus Instruments, Columbus, OH). We utilized a 0.5% sucrose/water solution for these tests, which has been shown to induce sucrose preference in C57BL/6 mice [31]. Mice were single housed for at least one week prior to being put into testing cages, which consisted of home cages fitted with two lixits located opposite and equidistant from the food hopper. Mice remained in testing cages during the 6 day-long experiment. For the first 4 days, mice were trained to use the dual lixit system with water in both source bottles. Following training, one source bottle was switched from water to a 0.5% sucrose solution and testing was continued for 2 additional days. Each day, at 07:00, liquid consumption data was collected and lixit positions were switched in order to determine if there was baseline preference for either lixit or for either side that liquid was delivered. If there was lixit preference at baseline we paired the sucrose solution with the less preferred lixit. While we were unable to correct for side bias in this paradigm, only 1 animal showed a side bias during testing. The percentage of sucrose consumed was expressed as a percentage of the total liquid consumption when reporting sucrose preference. Graphed data represent the percent change in preference for sucrose compared to water (Δ preference). Total water consumption during baseline testing days as well as during preference testing days was also calculated but no differences in water intake were observed between genotypes.

2.5. Cloning of adenoviral synaptophysin-mCherry vector (Ad-Syn-mCherry)

The synaptophysin-mCherry fusion protein (kindly provided by Andreas Jeromin) was cloned into the base adenoviral transfer vector pShuttle CMV-iN [32] using NotI and KpnI restriction sites. The vector was maxi-prepped and adenoviral stocks were generated as described previously [32].

2.6. Stereotaxic injection for tract tracing

Nts^{Cre} mice were administered presurgical analgesic, anesthetized using isofluorane and placed in a stereotaxic frame. After exposing the skull, a guide cannula with a stylet (Plastics One, Roanoke, VA) was lowered into the target regions. Coordinates to the LHA (from bregma) were A/P: −1.34, M/L −1.13 and D/V −5.20 in accordance with the atlas of

