Brief communication

MOLECULAR METABOLISM

Optogenetic activation of leptin- and glucose-regulated GABAergic neurons in dorsomedial hypothalamus promotes food intake via inhibitory synaptic transmission to paraventricular nucleus of hypothalamus

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ABSTRACT

Objective: The dorsomedial hypothalamus (DMH) has been considered an orexigenic nucleus, since the DMH lesion reduced food intake and body weight and induced resistance to diet-induced obesity. The DMH expresses feeding regulatory neuropeptides and receptors including neuropeptide Y (NPY), cocaine- and amphetamine-regulated transcript (CART), cholecystokinin (CCK), leptin receptor, and melanocortin 3/4 receptors. However, the principal neurons generating the orexigenic function in the DMH remain to be defined. This study aimed to clarify the role of the DMH GABAergic neurons in feeding regulation by using optogenetics and electrophysiological techniques.

Methods: We generated the mice expressing ChRFR-C167A, a bistable chimeric channelrhodopsin, selectively in GABAergic neurons of DMH via locally injected adeno-associated virus 2. Food intake after optogenetic activation of DMH GABAergic neurons was measured. Electrophysiological properties of DMH GABAergic neurons were measured using slice patch clamp.

Results: Optogenetic activation of DMH GABAergic neurons promoted food intake. Leptin hyperpolarized and lowering glucose depolarized half of DMH GABAergic neurons, suggesting their orexigenic property. Optical activation of axonal terminals of DMH GABAergic neurons at the paraventricular nucleus of hypothalamus (PVN), where anorexigenic neurons are localized, increased inhibitory postsynaptic currents on PVN neurons and promoted food intake.

Conclusion: DMH GABAergic neurons are regulated by metabolic signals leptin and glucose and, once activated, promote food intake via inhibitory synaptic transmission to PVN.

Keywords Dorsomedial hypothalamus; GABAergic neuron; Feeding; Leptin; Glucose; Optogenetics

1. INTRODUCTION

The dorsomedial hypothalamus (DMH) has been considered an orexigenic nucleus, since its lesion reduces food intake and body weight [1] and induces resistance against diet-induced obesity [2,3]. The DMH lesion also impairs food-entrainable circadian rhythms [4–6]. The DMH expresses feeding regulatory neuropeptides including neuropeptide Y (NPY) [7], cocaine- and amphetamine-regulated transcript (CART) [8], and prolactin-releasing peptide (PrRP) [9]. It also expresses various receptors, including leptin receptor [10], melanocortin 3/4 receptors (MC3/4) [11,12], Y1 receptor, Y5 receptor [13], and CCK receptor [14,15]. Region specific knock down and overexpression studies demonstrated that NPY neurons in DMH, which are GABAergic and leptin insensitive [7,16], play a role to promote food intake in rats [17–19], being consistent with the DMH-lesion studies. However, it was reported that the level of NPY expression is very low in mice fed with normal chow, questioning its physiological role, while it is increased in diet-induced obesity [20]. Hence, the principal orexigenic neuron in DMH under physiological conditions remains to be identified.

It was reported that the mice deficient in leptin receptor specifically in GABAergic neurons develop greater increases in food intake and body weight compared to the mice deficient in leptin receptor specifically in agouti-related protein (AgRP), proopiomelanocortin (POMC) or steroidogenic factor 1 (SF1) neurons [21]. These results suggested that GABAergic neurons including those in DMH could be a principal orexigenic neuron targeted by leptin [21].

In the present study, the role of GABAergic neurons in DMH in feeding regulation was analyzed using optogenetic and electrophysiological

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techniques. We found that DMH GABAergic neurons are hyperpolarized by leptin and depolarized by lowering glucose, and that their optogenetic activation elicits inhibitory synaptic transmission to the paraventricular nucleus of hypothalamus (PVN) where anorexigenic neurons are localized, and promotes food intake.

2. MATERIALS AND METHODS

2.1. Adeno-associated virus (AAV) 2 production

ChRFR-C167A, one of bistable variants of chimeric channelrhodopsins [22], was fused with Venus and cloned into AAV2-GAD1 promoter-WPRE-BGH-polyA vector. The AAV2 virus coded ChRFR-C167A-Venus was generated using GD1001-RV (Genedetect.com Ltd. Auckland, New Zealand). Titer of the virus was 1.1×10^{12} genomic particles/ml.

2.2. Animals

Male C57black6/J mice aged 8 weeks were maintained in a 12/12 h light/dark cycle. The AAV2-GAD1-ChRFR-C167A virus (50 nl/injection site) was injected stereotaxically to DMH at 1.4 mm caudal to the bregma in the midline, 0.2 mm lateral and 5.4 mm below the surface of the skull, under anesthesia with tribromoethanol (200 mg/kg). The optical fiber with 250 or 500 μm diameter was stereotaxically placed above DMH (at 1.4 mm caudal to the bregma in the midline, 0.5 mm lateral and 4.8 mm below the surface of the skull) or PVN (at 0.6 mm caudal to the bregma in the midline, 0.25 mm lateral and 4.4 mm below the surface of the skull). Mice were allowed to recover from the operation for 2 weeks. On the day of experiments, food was removed from cages at 15:30. The food was returned to cages at 19:30, and food intake at 0.5, 1, 2, 3, 6 h were measured. Exposure to blue laser (473 nm) followed by yellow (589 nm) laser (LUCIR, Tsukuba, Japan) was performed via optical fibers with 10 ms pulses, 50 Hz for 2 s, repeated every 5 s to the DMH or 3 s yellow pulse following 2 s blue pulse repeated every 10 s to the PVN for 3 h from 19:30 to 22:30. At the end of the experiments, the mice were perfused with 4% paraformaldehyde (PFA) in 0.1 M PB and the coronal sections of the hypothalamus were cut, to histologically verify the position of the virus infection and optical fiber.

The animal experiments for this study were carried out in a humane manner after receiving approval from the Institutional Animal experiment Committee of Jichi Medical University, and in accordance with the Institutional Regulation for Animal Experiments and Fundamental Guideline for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports and Technology.

2.3. Acute slice preparation

The brains were rapidly removed from C57BL/6J male mice infected AAV2-GAD1-ChRFR-C167A to DMH under anesthesia with tribromoethanol (200 mg/kg). The isolated brains were placed in ice-cold, carboxygenated (95% O_2 and 5% CO_2) high mannitol solution containing (in mM) 229 mannitol, 3 KCl, 6 $MgCl_2$, 0.5 $CaCl_2$, 1 NaH_2PO_4, 26 $NaHCO_3$, and 10 glucose at pH 7.4 with 0.5 μM tetrodotoxin (osmolarity; 300–305 mOsm). A block of tissue containing the hypothalamus was isolated and coronal slices (300 μm) were cut on a vibratome. Following recovery for 1–2 h, slices were moved to a recording chamber mounted on BX51WI upright microscope (Olympus) equipped with video-enhanced infrared-differential interference contrast (DIC) and fluorescence. Slices were perfused with a continuous flow of carboxygenated aCSF containing (in mM) 127 NaCl, 2.5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 1.23 NaH_2PO_4, 26 $NaHCO_3$, and 2.5–10 glucose at pH 7.4. Neurons were visualized with Olympus Optical 40× water-immersion lens.

2.4. Patch-clamp recording

Whole-cell current-clamp recordings were performed as previously reported [23]. Briefly, pipettes were used with 3–9 MΩ resistance after being filled with pipette solution. Pipettes were made of borosilicate glass (Narishige) using a PP-83 vertical puller (Narishige) or a Sutter micropipette puller (P-1000). The pipettes with 3–9 MΩ resistance after being filled with pipette solution were used. The composition of the pipette solution for current clamp recording was (in mM): 135 K-gluconate (for current clamp recording) or KCl (for IPSC recording), $MgCl_2$ 2, HEPES 10, EGTA 1.1, Mg-ATP 2.5, Na_2-GTP 0.3, and Na_2-phosphocreatine 10 at pH 7.3 with KOH (osmolarity; 290–295 mOsm). Axopatch 200B amplifier and Clampex 10 software (Axon Instruments) were used for data acquisition. Pclamp 10 (Axon Instruments) software was used for analysis. Liquid junction potential correction was performed off-line. Access resistance was continuously monitored during the experiments. Only those cells in which access resistance was stable (changes ~30%) were included in the analysis. The data was analyzed by Clamp fit 10 (Axon instruments) software and GraphPad Prism6 software. If a change of membrane potential was at least twice the standard deviation of membrane potential for 2 min before addition of agents, it was considered the response.

Irradiation was carried out using power LEDs (each from Lumileds, San Jose, CA) emitting either blue light (peak, 460–490 nm, LXHL-NB98) or yellow light (peak, 587–597 nm, LXHL-NL98) controlled by a regulator (SLA-1000-2, Mightex, Toronto, Canada). If the cumulative distribution of IPSC amplitude for 20 s after light exposure was significantly larger than that before light exposure by kolomogrv-semirnov test, it was considered the induction of light-evoked IPSCs.

2.5. Statistical analysis

Data are expressed as means ± s.e.m. Two-way ANOVA followed by Sidak multiple range tests was used for food intake experiments and one-way ANOVA followed by Dunnet multiple range tests for membrane potential experiments.

3. RESULTS

3.1. Optogenetic activation of GABAergic neurons in DMH promotes food intake

To selectively activate GABAergic neurons in DMH, AAV2 coded ChRFR-C167A-Venus under GAD1 promoter was infected to DMH. ChRFR-C167A, a bistable variant of chimeric channelrhodopsin, provides bimodal regulation: exposure to blue light (470 nm) induces long lasting opening, which is subsequently terminated by exposure to yellow light (592 nm) [22]. The mice expressing ChRFR-C167A-Venus in DMH GABAergic neurons were studied. Venus fluorescence was observed in DMH at 2 weeks after virus infection (Figure 1A). In acute slices including DMH under current clamp mode, the ChRFR-C167A-Venus expressing neurons were long lastingly depolarized by blue LED light exposure and repolarized by yellow LED light (Figure 1B). In these mice, food intake was measured with or without blue (473 nm) and yellow (589 nm) laser light exposure for 3 h via optical fiber inserted above DMH. Cumulative food intake at 2 and 3 h of light exposure was significantly greater than the corresponding values in mice without light exposure (Figure 1C). These data indicated that activation of DMH GABAergic neurons promoted food intake.

