The role of glial cells in feeding behaviors: History and progress report

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Abstract

The regulatory mechanisms of appetite are not fully understood. In this article, we introduce the history of experimental techniques related to feeding and the role of leptin in the hypothalamus. We also summarize previous studies of neural circuits related to feeding and the relationship of non-neuronal cells. Lastly, we report the progress of our study on the function of Olig2-positive cells in neuronal circuits for feeding.

Introduction

Appetite is an innate human feature maintained throughout life. Babies cry when they feel hungry, and even in the last stages of life, appetite-related behaviors are relatively maintained, although their neurological functions gradually decline with age. According to the World Health Organization (WHO), more than 1.9 billion people over 18 years old are overweight and over 650 million were obese in 2016. Excessive fat and sugar intake, in addition to less exercise, are major causes of obesity.

However, it is also important to elucidate neuronal mechanisms regulating food intake in order to understand appetite-related disorders and establish efficient clinical treatments for patients.

In this review article, we will first introduce the history of neuroscience research related to feeding behaviors, and recent progress in understanding glial cell functions in appetite control and metabolism. Lastly, we will briefly describe

current ongoing studies focusing on the role of the Olig2 transcription factor during the development of feeding-related neuronal circuits.

The history of studies exploring the neuronal mechanisms of feeding behaviors

Based on classical experiments that examined feeding behaviors of animals (rats or cats) after damaging or stimulating certain brain areas, the hypothalamus was identified as a center that controls appetite and feeding behaviors (Anand and Brobeck, 1951). However, these experiments were unable to exclude the possible involvement of nerve fibers originating from other brain areas. Thus, subsequent experiments specifically targeted cell bodies in the hypothalamus for lesioning or stimulation (Shimizu et al., 1987), which provided concrete evidence about the essential role of hypothalamic nuclei for appetite control.

In addition to neurophysiological experiments, researchers investigated the involvement of hormones and related substances as possible mediators for appetite control depending on the activity of hypothalamic neurons.

Leptin is the obese (ob) gene product that is mutated in spontaneously mutant ob/ob mice (Zhang et al., 1994) and is a hormone secreted from adipose tissue. Although the discovery of leptin was expected to solve obesity, obesity caused by mutation of the ob gene is uncommon (Montague et al., 1997). The majority of cases of obesity without genetic abnormalities are associated with leptin resistance (Caro et al., 1996). These studies suggested that leptin plays a role in maintaining homeostasis and the existence of functions for that balance.

The primary target of leptin is the arcuate nucleus of the hypothalamus, which contains diverse classes of neurons. Leptin activates proopiomelanocortin(POMC) neurons but suppresses agouti-related peptide (AgRP) and neuropeptide Y (NPY) neurons, resulting in the suppression of food intake. However, as leptin also has diverse functions outside the hypothalamus, it is important to identify the neuronal circuitry regulating food intake. Recent advances in experimental

techniques enabled the examination of the causal relationship between neuronal activity and phenotypic outcomes.

Optogenetics and chemogenetics are two major examples of such technical advancements to directly manipulate neuronal activity. Optogenetics utilizes a light-sensing channelrhodopsin from green algae, which once expressed, can be used to stimulate specific neurons. The advantage of this method is temporal precision of stimulation by altering blue light illumination. Chemogenetics, such as DREADD (Designer Receptors Exclusively Activated by Designer Drugs), utilizes genetically engineered receptors that can be activated by designed ligands. In contrast to optogenetics, chemogenetics does not need intracranial implants for the control of neuronal activation, which significantly interferes with animal motility. These innovative techniques enabled us to clarify the role of specific neuronal circuits in specific behaviors (Krashes and Kravitz, 2014).

The study performed by Domingos et al. (2011) provided novel insight into appetite control using optogenetics. Experiments to investigate food preferences using mice were conventionally performed in subjective manners such as observing facial expressions or counting the number of licks of sweet-tasting water. By using optogenetics in combination with conventional food preference tests, leptin was demonstrated to be responsible for taste-dependent behaviors through dopaminergic neurons (Domingos et al., 2011). This study suggested that optogenetics and chemogenetics are powerful tools for clarifying leptin functions in a specific neuronal circuit.

Glial cells and metabolism

Most previous studies on feeding-related neural circuits focused on neurons, whereas recent studies are focusing on the roles of non-neuronal cells, such as ependymal cells and glial cells, in the control of food intake and metabolism. As such, functional contributions of non-neuronal cells to neuronal circuits are considered to control energy consumption and appetite.

A previous study on glial cells and neural circuits for feeding suggested that

tanycytes located at the median eminence (ME) of the hypothalamus are involved in selective leptin incorporation into the brain (Balland et al., 2014). Tanycytes are a subtype of ependymal cells that share characteristics with glial cells. They reported that leptin from the periphery is transported to the cerebrospinal fluid in the third ventricle via tanycytes, where it regulates the activity of AgRP or POMC neurons in the hypothalamus. Transportation of leptin through tanycytes depends on ERK activity. This study revealed that tanycytes play a central role in leptin-dependent appetite control.

In addition, the disruption of NG2-positive glial cells in the ME caused body weight increases and obesity in mice (Djogo et al., 2016). NG2-positive glial cells are oligodendrocyte precursors, but this study suggested an additional role of this type of glial cell in the control of nutritional status, possibly mediated by leptin-dependent neuronal circuits for morphological reasons. Ablation of NG2 cells disrupted the neuronal fibers of leptin receptor-positive neurons, suggesting that NG2 cells in the ME are required for maintaining leptin-dependent neuronal circuits. These reports support the importance of glial cells in regulating feeding behavior.

Possible contribution of Olig2 lineage cells in neuronal circuits for feeding control

Identification of the developmental origins of neuronal circuits will shed light on the regulatory mechanisms of appetite control, which may be translated to clinical treatment for obesity and overeating. Following Djago's study, we became interested in the relationship between the feeding-related neural circuits and Olig2, an essential transcription factor for NG2-glial cell generation (Ono et al., 2008). Olig2 is expressed in neural progenitor cells in the developing central nervous system and Olig2-positive progenitors have the potential to differentiate into multiple cell types, including neurons and glial cells. Therefore, whether Olig2-positive progenitors function in the leptin-dependent neural circuits is of interest.

To clarify the contribution of Olig2 lineage cells to hypothalamic neuronal circuits, we performed genetic labeling by crossing Olig2-CreER (tamoxifen-inducible estrogen receptor Cre) mice (Takebayashi et al., 2002) with floxed-GFP reporter mice, enabling the permanent labeling of Olig2 lineage cells at specific embryonic stages in a tamoxifen-dependent manner. We examined the distribution and types of labeled cells in the hypothalamus of young adult mice. Approximately 70-80% of the cells labeled at fetal stages differentiated into astrocytes in the hypothalamus of mature mice. In contrast, the proportion of neurons and oligodendrocytes among labeled cells was lower than that of astrocytes (approximately 10% and less than 10%, respectively; Nakamori et al., unpublished observation).

Next, we investigated the role of Olig2 lineage cells in feeding behaviors. c-Fos is a transcription factor whose expression is rapidly induced in response to neuronal activation. The expression of c-Fos changes under stress conditions such as fasting or nutritional alterations (Kim et al., 2014). According to this report, the rate of c-Fos-positive POMC neurons increased after the administration of leptin. In addition, the rate of c-Fos-positive AgRP neurons increased after 18 hours of fasting. As relatively few neurons were labeled after Cre-mediated recombination in our study, we were unable to observe c-Fos/GFP-double-positive neurons in our experiments. In addition, few GFP-positive astrocytes expressed c-Fos. Thus, currently, we do not have direct evidence for the involvement of Olig2 lineage cells in feeding behavior.

Conclusion and perspectives

The contribution of Olig2 lineage cells to neuronal circuits for feeding behavior has not been confirmed. Our current study is focusing on adult mice; however, we need to investigate earlier stages because leptin and its receptors also function during embryogenesis (Baquero et al., 2014). It was previously suggested that leptin receptors are more highly expressed in NPY/AgRP/GABA (NAG) neurons than in proopiomelanocortin (POMC) neurons in early postnatal stages, and

promote the rapid growth of neonatal mice. Of note, such promoting effects of leptin on growth are absent in adult mice.

Although obesity is considered a disease and is treated in the field of internal medicine, interdisciplinary collaboration, including neurological and physiological points of view, is indispensable for obesity treatment. The hypothalamus plays a central role in the control of feeding behaviors.

Further investigations focusing on the role of different cells, including glial cells, are necessary to clarify the regulatory mechanisms of appetite control, which will improve our understanding of feeding disorders and obesity.

Acknowledgment

This study was supported by a Grant-in-Aid for scientific research provided by MEXT and JSPS.

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