学 位 論 文 の 要 旨

氏名　中 村 佐 和 子

学 位 論 文 名　Amygdaloid Axons Innervate Melanin-Concentrating Hormone- and Orexin-Containing Neurons in the Mouse Lateral Hypothalamus

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著 者 名　Sawako Nakamura, Toshiko Tsumori, Shigefumi Yokota, Tatsuro Oka, Yukihiko Yasui

論 文 内 容 の 要 旨

INTRODUCTION

Melanin-concentrating hormone (MCH)- and orexin (ORX)-containing neurons in the lateral hypothalamus (LHA) have been considered essential in regulating food intake and body weight. On the other hand, the amygdala has been suggested to influence feeding behavior through its projection to the LHA. In addition, it has been revealed that the LHA receives projection fibers from the central amygdaloid nucleus (CeA), which is one of the main output nuclei of the amygdala. Judging from the above, it seems quite probable that MCH- and ORX-containing neurons in the LHA are under the direct influence of the CeA in the control of feeding behavior. However, the question whether or not these neurons receive monosynaptic inputs from the CeA remains unanswered.

With respect to the neurotransmitter, γ-aminobutyric acid (GABA)-immunoreactive (ir) CeA terminals have been observed in several forebrain regions of the rat including the parastrial nucleus and posterior lateral hypothalamus. However, there have been no studies to examine whether or not the mouse CeA-LHA projection is GABAergic.

In the present study, we first provide definitive evidence for the existence of a monosynaptic pathway from the CeA to MCH- and ORX-containing neurons in the LHA, and then examine whether or not CeA axon terminals in the mouse LHA are immunoreactive for glutamic acid decarboxylase (GAD), an enzyme for conversion of glutamic acid to GABA.

MATERIALS AND METHODS

Experiments were carried out on female C57BL/6J mice weighing between 20 and 30 g, which were anesthetized with intraperitoneal injection of chloral hydrate (350 mg/kg).

Three mice were perfused transcardially, and the brains were cut serially into frontal sections at 40 μm thickness on a freezing microtome. Subsequently, MCH-ir or ORX-ir neurons in the hypothalamus were detected immunohistochemically using anti-MCH or anti-ORX-A antibody.

Injections of biotinylated dextranamine (BDA) into the CeA were made stereotaxically by iontophoresis in 15 mice. After 5-7 days of survival, the mice were perfused transcardially, and the brains were cut serially into frontal sections at 40 μm thickness on a freezing microtome. BDA-labeled axons were visualized with avidin-biotin-peroxidase complex (ABC) and stained brown by using diaminobenzidine (DAB). Then MCH-ir or ORX-ir neurons were detected immunohistochemically and stained dark blue by using VECTER SG. In the electron microscopic experiment, BDA was first visualized with DAB. Subsequently, the sections were treated with primary antibodies and then with secondary antibodies. After silver-gold intensification of DAB reaction product of BDA was performed, the sections were incubated in the ABC solution, and then the second DAB reaction was done for visualization of MCH-ir or ORX-ir neurons. Specimens in which there was a good overlapping distribution of BDA-labeled fibers and MCH-ir or ORX-ir neurons were cut from the LHA region, and then examined under an electron microscope.

Injections of BDA into the CeA were made in 5 mice. After perfusion, the brains were removed, dissected into 5-mm-thick blocks and sectioned 40 μm thick in the frontal plane on a vibrating microtome. After detection of GAD immunoreactivity by using a preembedding immunogold-silver method, BDA-labeled axons were visualized with ABC method. Subsequently, the specimens were processed for electron microscopic observation.

RESULTS AND DISCUSSION

The present study is the first to examine the distribution patterns of MCH-ir and ORX-ir neurons in the mouse hypothalamus, indicating that the results are nearly the same as those described in the rat. Both MCH-ir and ORX-ir neurons were localized nearly exclusively in the tuberal hypothalamus. MCH-ir neurons were distributed predominantly in the incerto-hypothalamic area, dorsolateral part of the LHA and perifornical region, with some in the dorsomedial nucleus and a few in the zona incerta. ORX-ir neurons were distributed predominantly in the dorsal part of the LHA, with some in the dorsomedial nucleus and perifornical region, and a

few in the ventral part of the LHA.

In the mice that were successfully injected with BDA in the CeA, the sections including the hypothalamus were immunostained for MCH or ORX. In these mice, a dense plexus of BDA-labeled fibers was found in the dorsolateral part of the LHA just medial to the internal capsule as well as to the subthalamic nucleus. Some labeled axons were present in the zona incerta, and a few in the paraventricular, dorsomedial and ventromedial nuclei, and perifornical region. On the other hand, the distribution patterns of MCH-ir and ORX-ir neurons in the hypothalamus were the same as those described above. Thus, the overlapping distribution of BDA-labeled fibers and MCH-ir or ORX-ir neurons was found predominantly in the dorsolateral part of the LHA just medial to the subthalamic nucleus, and additionally in the dorsolateral part of the LHA just medial to the medial margin of the internal capsule. In these areas, BDA-labeled boutons were often closely apposed to cell bodies and dendrites of the MCH-ir or ORX-ir neurons. Under the electron microscope, almost all the CeA terminals labeled with BDA made symmetrical synaptic contacts with somata and dendrites of the LHA neurons, some of which were immunoreactive for MCH or ORX.

In the mice injected with BDA into the CeA, almost all the BDA-labeled terminals in the dorsolateral part of the LHA were identified to be immunoreactive for GAD, which is a marker for GABAergic neurons, and these terminals contained large numbers of pleomorphic clear vesicles and formed symmetrical synaptic contacts with somata or dendrites of the LHA neurons.

Previous studies have shown that lesions of the amygdala result in various changes of feeding and body weight, and it has been suggested that the amygdala regulates feeding behavior in part through its projection to the LHA. The monosynaptic CeA connection with the MCH- and ORX-containing LHA neurons revealed here may represent an anatomical substrate for this amygdaloid regulation.

CONCLUSION

The present study indicates that MCH- and ORX-containing neurons in the mouse LHA receive CeA fibers and symmetrical synapses are made between these neurons and fibers, and that CeA axon terminals are immunoreactive for GAD, suggesting that the MCH- and ORX-containing LHA neurons may be under the inhibitory influence of the GABAergic CeA neurons.