学 位 論 文 の 要 旨

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学 位 論 文 名　Posterior Lateral Hypothalamic Axon Terminals Are in Contact With Trigeminal Premotor Neurons in the Parvicellular Reticular Formation of the Rat Medulla Oblongata

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論 文 内 容 の 要 旨

INTRODUCTION

The hypothalamus has been well known to play a critical role in a variety of functions, including not only the control of endocrine and autonomic functions but also motivated and emotional behaviors. It is also known that electrical stimulation of the hypothalamus induces attack or defense responses in which opening and closing movement of jaws is one of the obvious elements. The motor behavior of the jaw muscles is elicited by the firing of motoneurons which exist mainly in the motor trigeminal nucleus (Vm). It has been reported that the parvicellular reticular formation (RFp) in the lower brainstem contains many premotor neurons that project directly to the Vm, and receives descending fibers from the lateral hypothalamic area (LHA). Judging from the above, it seems likely that the LHA-RFp-Vm pathway exists and plays a crucial role in the control of emotion-related jaw movements. However, there have been no studies to examine whether or not RFp neurons that send their axons to the Vm receive direct inputs from the LHA.

In this communication, we first examined the distribution field of RFp-projecting neurons in the hypothalamus by using retrograde tracing technique, and then provided definitive evidence for the existence of a disynaptic pathway from the LHA to the Vm via the RFp by using combined anterograde and retrograde tracing technique. In this set of experiments, we found that the synapses between the LHA axon terminals and the Vm-projecting RFp neurons are of asymmerical type, suggesting that these synapses are excitatory. Therefore, we finally examined whether or not LHA axon terminals in the RFp are immunoreactive for vesicular glutamate transporter 2 (VGLUT2), a much better marker for glutamatergic excitatory neurons, by using anterograde tracing combined with preembedding immunolabeling techniques.

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats weighing 250 to 330 g under general anesthesia with intraperitoneal injection of chloral hydrate (350 mg/kg).

Injections of cholera toxin B subunit (CTb) into the RFp of the medulla oblongata were made stereotaxically by iontophoresis in 7 rats. After 7 days survival, the rats were perfused transcardially with saline, followed by a solution of 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.3). The brains were removed and post-fixed in a solution of 4% paraformaldehyde in 0.1 M PB and saturated with 20% sucrose in 0.1 M PB. The brains were cut into serial frontal sections at 50 μm thickness on a freezing microtome. CTb-labeled neurons were detected immunohistochemically and stained brown by using diaminobenzidine (DAB) as a chromogen.

Ipsilateral injections of CTb into the Vm and biotinylated dextran amine (BDA) into the posterior lateral hypothalamus (PLH) were made in 13 rats. After 5-7 days of survival, the animals were perfused with saline, followed by a solution composed of 4% paraformaldehyde and 0.2% glutaraldehyde in 0.1 M PB. The brains were post-fixed with 4% paraformaldehyde in 0.1 M PB, and saturated with 20% sucrose in 0.1 M PB. Serial frontal sections of the brainstems were cut at 40 μm on a vibrating microtome. In a one-in-two series of sections for the light microscopic study, BDA-labeled axons were visualized with avidin-biotin-peroxidase (ABC) and stained dark blue to black by using DAB and nickel ammonium sulfate as a chromogen. Subsequently, CTb–labeled neurons were visualized with above-mentioned procedures. In another series for the electron microscopic study, BDA-labeled terminals were detected with ABC, and then silver-gold-intensification of DAB reaction products of BDA was performed. CTB-labeled neurons were detected as mentioned above. Specimens were cut out from the RFp region, post-fixed with osmium tetroxide, stained with uranyl acetate, dehydrated, cleared in propylene oxide, and then embedded flat in Epon. Subsequently, ultrathin sections were cut, stained with lead nitrate, and then examined under an electron microscope.

BDA injection was made into the PLH in 7 rats. After 7 days survival, the 4 out of 7 rats were perfused transcardially with saline, followed by a solution composed of 4% paraformaldehyde and 0.05% glutaraldehyde in 0.1 M PB. The brains were postfixed with 4% paraformaldehyde in PB. The remaining 3 rats were perfused with saline, followed by a solution composed of 2% paraformaldehyde and 3.75% acrolein in 0.1 M PB, and a solution of 2% paraformaldehyde in PB. The brains were postfixed with 2% paraformaldehyde in PB. Subsequently, the brains were sectioned 40-μm thick in the frontal plane on a vibrating microtome. VGULT2 was detected immunohistochemically, and silver-gold-intensification of immuno-reaction products was performed. BDA-labeled terminals were detected with ABC. After visualization of BDA, the specimens were processed for electron microscopic observation as mentioned above. Finally, the sections were stained with lead citrate and then examined under an electron microscope.

RESULTS AND DISCUSSION

In the rats injected with CTb into the RFp of the mdulla oblongata, CTb-labeled neurons were observed in the hypothalamus, bilaterally with an ipsilateral dominance. At the rostral level of the hypothalamus, some labeled neurons were found in the paraventricular nucleus. At the midlevel of the hypothalamus, many labeled neurons were scattered in the LHA. In the more caudal sections, a distinct population of labeled neurons was found in the PLH just medial to the subthalamic nucleus, with some labeled neurons around the PLH. At the caudal level of the hypothalamus, moderate numbers of labeled were still found in the PLH.

In the rats that received combined injections of BDA into the PLH and CTb into theVm, BDA-labeled axons around or on CTb-labeled neurons were seen throughout the entire rostrocaudal extent of the RFp ipsilateral to the injection sites, which was most prominent in the RFp region just ventral to the nucleus of the solitary tract. In this RFp region, many CTb-labeled cell bodies were embedded in the plexus of BDA-labeled axons, and BDA-labeled boutons were in close apposition to somata or dendrites of the CTb-labeled neurons. When we examined the target sites of 85 BDA-labeled axon terminals forming synapses with RFp neurons under electron microscope, approximately 54% (n = 46) of them were found to make synaptic contacts with CTb-labeled RFp neurons. The contacts were both axosomatic (n = 3) and axodendritic (n = 43). These synapses were of asymmetrical type.

In the rats injected with BDA into the PLH, almost all the PLH terminals with BDA were identified to be immunoreactive for VGLUT2. In the rats perfused with a mixture of 2% paraformaldehyde and 3.75% acrolein in 0.1 M PB, the labeling of VGLUT2 immunoreactivity was much better than that in the rats that were perfused with a mixture of 4% paraformaldehyde and 0.05% glutaraldehyde in 0.1 M PB. The BDA-labeled PLH terminals with VGLUT2 immunoreactivity formed asymmetrical synapses predominantly with dendrites and additionally with somata of the RFp neurons.

Here we indicated that RFp neurons receiving PLH fibers send their axons to the Vm and asymmetrical synapses are made between these PLH fibers and Vm-projecting RFp neurons, and that the PLH axon terminals in the RFp are immunoreactive for VGLUT2. Our previous study demonstrated the existence of the pathway from the central amygdaloid nucleus (CeA) to the Vm via the RFp, and suggested that the CeA-RFp-Vm pathway may be responsible for the control of jaw movements closely related to emotional behavior. Furthermore, it has been reported that there are reciprocal connections between the CeA and the PLH. These data suggest that the PLH-RFp-Vm and CeA-RFp-Vm pathways work in concert for producing jaw movements during emotional behavior.

CONCLUSION

The present study demonstrates that PLH-RFp-Vm pathway exists and suggests that the PLH exerts a glutamatergic excitatory action upon RFp neurons sending their axons to the Vm for the control of emotion-related jaw movements.