# 学 位 論 文 の 要 旨

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| 学位論文名 | A Disynaptic Pathway From the Central Amygdaloid Nucleus to the Paraventricular Hypothalamic Nucleus via the Parastrial Nucleus in the Rat |
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## 論 文 内 容 の 要 旨

### INTRODUCTION

The central amygdaloid nucleus (CeA) has been considered to play an important role in the control of endocrine and autonomic functions, in part through its projection to the hypothalamic paraventricular nucleus (PVH). Direct projections from the CeA to the PVH are quite sparse. It has been demonstrated that the CeA densely innervates the bed nucleus of the stria terminalis (BST), which in turn projects to the PVH, suggesting that the BST may act as a relay between the CeA and PVH. However, a previous dual tract-tracing study has indicated that PVH-projecting BST neurons are occasionally apposed by the CeA axon terminals.

In the course of experiments dealing with the CeA-forebrain projections, we noticed that moderate numbers of CeA fibers terminated in the parastrial nucleus (PS), which has been considered to be included in the BST and known to contain many neurons projecting to the PVH. Judging from the above, it is quite likely that there exists a disynaptic pathway from the CeA to the PVH via the PS. In the present study, we provide definitive evidence for the existence of this pathway, and then examine whether or not CeA axon terminals in the PS are immunoreactive for γ-aminobutyric acid (GABA).

## MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing between 250 and 300 g. All surgical procedures were performed under general anesthesia with intraperitoneal injection of chloral hydrate (350 mg/kg).

Ipsilateral injections of cholera toxin B subunit (CTb) into the PVH and biotinylated dextranamine (BDA) into the CeA were made stereotaxically by iontophoresis. After 5-7 days of survival, the rats were deeply reanesthetized and perfused transcardially, and the brains were cut serially into frontal sections at 50 μm thickness on a freezing microtome. A one-in-two series of forebrain sections were used for histochemical visualization of BDA-labeled fibers and immunohistochemical visualization of CTb-labeled neurons. In another series of forebrain sections, BDA-labeled fibers were visualized histochemically with silver-gold intensification of diaminobenzidine, and CTb-labeled neurons were visualized immunohistochemically. Specimens in which there was a good overlapping distribution of BDA-labeled fibers and CTb-labeled neurons were cut out from the PS region, and then examined under an electron microscope.

A retrograde tracer, CTb or an anterograde tracer, BDA was injected into the PS, where good overlapping distribution of CeA fibers and PVH-projecting neurons had been found, in 7 or 8 rats, respectively. Frontal sections of the brains were obtained, and then immunohistochemical visualization of CTb or histochemical visualization of BDA was performed.

Injections of BDA into the CeA were made in 3 rats. After perfusion, the brains were removed, dissected into 5-mm thick blocks and sectioned 30-μm thick in the frontal plane on a vibrating microtome. After visualization of BDA, the specimens were processed for electron microscopic observation with the postembedding immunogold technique for revealing GABA immunoreactivity.

## RESULTS AND DISCUSSION

In the rats that received ipsilateral injections of CTb into the PVH and BDA into the CeA, we

observed a light overlap of the distribution of CeA fibers and PVH-projecting neurons in the anterior medial, ventral medial and preoptic subnuclei of the BST, as reported by a previous study. Here we demonstrated for the first time that the PS displayed greater overlapping distribution of CeA fibers and PVH-projecting neurons: moderate numbers of CeA fibers innervated the PS where large numbers of PVH-projecting neurons existed, and that the CeA axon terminals made symmetrical synaptic contacts with somata and dendrites of the PS neurons sending their axons to the PVH. We further indicated that the PS received CeA fibers predominantly from the lateral part and additionally from the medial part, and that all of the PVH subdivisions contained moderate to large numbers of PS axon terminals. Judging from these data, it seems quite probable that the PS serves as a relay between the CeA and the PVH.

In the PS region, almost all the CeA terminals labeled with BDA were identified to be immunoreactive for GABA, and these terminals contained large numbers of pleomorphic clear vesicles and formed symmetrical synaptic contacts with somata or dendrites of the PS neurons. The PS has been reported to contain many GABA-immunoreactive neurons, some of which send projection fibers to the PVH. Taken together, these data suggest that the indirect CeA-PVH pathway relayed at the PS may exert disinhibitory influence on PVH neurons

## CONCLUSION

There is a good overlapping distribution of CeA fibers and PVH-projecting neurons in the PS, and symmetrical synapses are made between these fibers and neurons. Furthermore, the CeA axon terminals in the PS are immunoreactive for GABA. Along with previous findings, these results suggest that a GABAergic CeA-PS-PVH pathway may disinhibit PVH neurons.