（様式第１３号）

学 位 論 文 要 旨

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題目: Localization and functional characterization of glutamate-gated chloride channels in the housefly *Musca domestica*
（イエバエにおけるグルタミン酸作動性クロルイオンチャネルの局在及び機能解析）

L-Glutamic acid (hereafter glutamate) and γ-aminobutyric acid (hereafter GABA) are the major neurotransmitters. In invertebrates such as insects, these neurotransmitters mediate inhibitory neurotransmission by acting on specific ionotropic receptor (glutamate also mediates excitatory neurotransmission in invertebrates). Inhibitory glutamate receptor, glutamate-gated chloride channels (GluCls), are only present in invertebrates. Invertebrate GABA-gated chloride channels (GABACls) differ from mammalian ionotropic $GABA_A$ receptors in their subunit composition. Thus, these channels are the target of insecticides and anthelmintics; many studies about pharmacological properties of these channels using electrophysiological techniques have been performed. However, there are few reports about comparison between two similar ionotropic receptors in terms of physiological roles. Therefore in this study, I investigated channels gene expression levels and channel localization as a basis to understand differences in physiological roles using housefly, *Musca domestica*.

First, I investigated gene expression levels of encoding housefly GluCl (*MdGluCl*) and Rdl (*MdRdl*) by quantitative real-time PCR. Both channel genes highly expressed in the adult head. Interestingly, MdGluCl expression level was higher than MdRdl in the adult legs. Next, I investigated localization of both channels in the housefly using specific antibodies. MdGluCl staining was located in the optic lobe such as lamina, medulla, retina basement membrane and pigment cells in the adult head. MdRdl was located in medulla, lobula and lobula plate in the optic lobe, and antennal lobe and mushroom body where correlate with olfactory learning and memory. Differences in localization were observed in the adult thorax. MdGluCl was located in the cell surface of motor neuron cell bodies, whereas MdRdl was distributed in the neuropile of motor neurons in the thoracic ganglion. In addition, anti-MdGluCl immunoreactivity was distributed in the leg. These findings revealed that two similar inhibitory receptors work in different tissues.

MdGluCl gene has three splice variants termed MdGluClA, B and C at exon 3 generated by alternative splicing. In addition, MdGluCl gene has four RNA editing sites. I performed quantitative PCR and two-electrode voltage clamp (TEVC) to investigate gene expression and pharmacological properties. MdGluClA and B transcripts highly were expressed in the adult head, while MdGluClC transcript was expressed in the adult head and in the peripheral regions such as leg and abdomen. These findings suggested

that three splice variants differ in their expression levels and expression patterns; MdGluCl channels expressed in the leg may be MdGluClC channels. In the functional characterization, there was no significant difference in sensitivity to glutamate and the activator ivermectin B_{1a} when the variants were singly- or co-expressed in *Xenopus* oocytes. In contrast, MdGluClA and B channels were more sensitive to GABA receptor blockers fipronil and picrotoxinin (PTX), than MdGluClC channels. These results suggested that MdGluClA and B channels expressed in the central nervous system in the brain are sensitive to channel blockers, and MdGluClC expressed in the peripheral tissues.

Antagonists that specifically act on GluCls are not known. Therefore, I tested some compounds known as GABACl noncompetitive antagonists against MdGluCl. Fipronil and PTX inhibited glutamate-induced currents, but sensitivities to GluCls were lower than to GABACls. Bicyclophosphorothionates TBPS, PS-14 and 1HEPS showed weak inhibition of three MdGluCl variants. Compounds which selectively act on GluCls were not identified in this study.

Both GluCls and GABACls mediate inhibitory neurotransmission in invertebrates, their localizations are different. Therefore, GluCls play different roles in the housefly body from GABACls. In addition, *MdGluCl* gene has three splice variants differ in their expression levels and expression sites. These findings suggest that MdGluCl regulates inhibitory neurotransmission in different body part and tissues by splice variants.