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学 位 論 文 要 旨

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題目: Ivermectin action on glutamate- and GABA-gated chloride channels
（グルタミン酸およびGABA作動性クロルイオンチャネルに対するイベルメクチンの作用）

L-Glutamic acid (Glu) and γ-aminobutyric acid (GABA) are the major neurotransmitters that exert both excitatory and inhibitory effects on neurotransmission in vertebrates. In invertebrate, Glu and GABA exert inhibitory effects by binding Glu- and GABA-gated chloride channels (GluCls, GABACls), although Glu also has excitatory effects. GluCls are present only in invertebrates and invertebrate GABACls differ from vertebrate GABA receptors in their subunit composition; therefore, these channels are major targets of insecticides and anthelmintics.

Ivermectin (IVM) is synthesized from avermectins that are produced by *Streptomyces avermitilis*, and IVM reportedly exerts insecticidal activities by acting on GluCls. The X-ray crystallographic analysis and site-directed mutagenesis studies of GluCls suggested that IVM binds at the transmembrane interface between TM1 to TM3. However, identification of the IVM-binding site by chemical means has yet to be achieved.

In this study, photoreactive IVM probes were synthesized and it was examined whether GluCls were photolabeled by the photoreactive probes. First, three photoreactive IVM probes (**PP1-3**), in which the dioleandrosyl moiety of IVM was replaced with different photoreactive substituents, were synthesized, and these probes were examined for their affinity for *Haemonchus contortus* GluCls (Hco-AVR-14B GluCls) and *Bombyx mori* GluCl (*Bombyx*/*D*-GluCl) using [^{3}H]IVM B_{1a} competition assays. Furthermore, the ability of these probes to activate GluCls was examined by two-electrode voltage clamp (TEVC) methods with Hco-AVR-14B GluCls. Of the three PPs, **PP2** was superior in both affinity and function at Hco-AVR-14B-GluCls. Next, **IodoPP2** was synthesized to prepare **[^{125}I]IodoPP2**, and it was confirmed that the affinity of **IodoPP2** does not differ from that of **PP2** using the [^{3}H]IVM B_{1a} assays with Hco-AVR-14B GluCls. Finally, **[^{125}I]IodoPP2** was synthesized from **IodoPP2** using the chloramine-T method. The photoaffinity labeling of Hco-AVR-14B-GluCls solubilized from oocyte membranes with the radiolabeled probe were performed. However, no specific cross-linked band of Hco-AVR-14B-GluCls was detected in the SDS gel.

IVM acts at various ion channels, but the molecular mechanism of action of IVM is not well defined. Therefore, the action of IVM was examined on the housefly (*Musca*

domestica) GluCls and GABACls. IVM elicited an irreversible response when applied alone to both channels. In this study, IVM itself induced currents in *Musca* GABACls by the administration for 3 min. In addition, *Musca* GluCls showed high sensitivity to IVM with 184-fold greater EC_{50} than *Musca* GABACls. The IVM potentiation and inhibition of currents induced by different concentration of GABA were examined in *Musca* GABACls. IVM potentiates currents induced by concentrations of GABA below its EC_{50}, whereas it inhibits current induced by concentrations of GABA above its EC_{50}. It was confirmed that IVM potentiation of currents induced by the EC_5 of Glu or GABA and the IVM inhibition of currents induced by the EC_{90} of Glu or GABA were observed in *Musca* GluCls and GABACls. The sensitivity of IVM in both potentiation and inhibition in GluCls was higher than those in GABACls, indicating that IVM's primary target is GluCls as GluCls are more sensitive to IVM than GABACls in three actions.

Substitution of an amino acid residue at the 36' position of GluCls, and GABACls resulted in significantly reduced levels or loss of IVM triple actions. Therefore, the glycine at the 36' position in TM3 is likely among the most important residues for the action of IVM. It is likely that these three actions result from the interaction of IVM with amino acid residues in the transmembrane intersubunit crevice.