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Studies on Neurotransmitter Receptors and Their Ligands*

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Structure-activity relationship studies of γ-aminobutyric acid (GABA) antagonists revealed that there are structural differences between the antagonist binding sites of houseflies and rats. The results point to the feasibility of the development of safe insecticides targeting insect GABA receptors. Studies with cockroach nerve cords demonstrated unique actions of dinotefuran and benzylidene anabaseines on insect nicotinic acetylcholine (ACh) receptors. Tyramine (TA) was shown to be a bioactive amine in the silkworm, and the structures of TA receptor ligands were disclosed.

Keywords: GABA, acetylcholine, octopamine, tyramine, receptor, ligand.

1. Introduction

The transmission of information between cells is achieved through the binding of chemical messengers to the membrane receptors. Chemical messengers in the nervous system include neurotransmitters, neuromodulators, and neurohormones. The neurotransmitter receptors are important sites of action of insecticides. This paper briefly describes the results of the studies we undertook to explore the potential of the GABA, ACh, and TA receptors as targets for insecticides.

2. GABA Receptors

Our studies on GABA receptors began, in the mid-1970s, with studies to elucidate the mode of action of bicyclophosphates (BPOs). BPOs are unique caged organophosphorus compounds that show high, acute toxicity to mammals. BPOs are low in reactivity, and are not acetylcholinesterase inhibitors. Quantitative structure-activity relationship (QSAR) studies showed that the toxicity is greatly dependent on the properties of the substituent at the 4-position. The substituent conferring the highest toxicity was found to be a *t*-butyl group, suggesting that the fitting of the substituent into some sort of receptor induces the toxicity. To identify the receptor, we prepared radioligands to label the rat brain receptor, *i.e.*, [^{14}C]4-*t*-butyl BPO and [^{3}H]4-*n*-propyl BPO. We used [^{3}H]4-*n*-propyl BPO to demonstrate that the binding protein is the GABA receptor. Electrophysiological studies showed that BPOs inhibit miniature inhibitory junction potentials in the longitudinal muscles of earthworms, which are muscles that are innervated by GABAergic neurons. These findings indicated that BPOs act as antagonists of the GABA receptors. 4-Substituted BPOs exhibit high mammalian toxicity, but low insecticidal activity. To discover analogues that display the reverse selectivity, we synthesized bicyclophosphorothionates (BPSs) that had substituents at both the 3- and 4-positions. The introduction of an appropriate substituent into the 3-position led to noncompetitive GABA receptor antagonists that showed a *ca.* 50-fold selectivity for the housefly *versus* rat GABA receptors, as well as high insecticidal activity.

A variety of noncompetitive GABA receptor antagonists, such as γ-BHC, cyclodienes, and bicycloorthocarboxylates (BOCs), were disclosed in the 1980s. γ-BHC and cyclodienes seemed to have structural moieties that are similar to the long known antagonist picrotoxinin, and therefore they are speculated to bind to the same binding site. However, BPOs, BPSs, and BOCs apparently belong to a different group of compounds. To understand why structurally diverse compounds elicit the same mode-of-action, we synthesized hybrids that bridged the gap between both groups of compounds, dioxatricyclododecenes (DTD), and determined their insecticidal activities and receptor affinities. Based on their structure-activity relationships, we then proposed a 4-subsite model for the binding site where a

* See Part II for the full Japanese article.

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variety of antagonists interact with two to three different subsites in overlapping orientations, and then verified the hypothetical model by three-dimensional QSAR analysis. The results revealed structural differences between the housefly and rat binding sites. The 4-subsite model remains to be verified by the structural analysis of GABA receptors. While conducting this research, we also discovered several novel types of antagonists.

In addition to being found in synthetic compounds, GABA antagonists are found in the natural world. Picrotoxin, a 1 : 1 mixture of picrotoxinin and picrotin, is the best known of the GABA antagonists, and is isolated from plants. We found that picrodendrins, a recently isolated group of picrotoxane terpenoids, act as antagonists at fly and rat GABA receptors, and that picrodendrin Q is more potent than picrotoxinin. Several picrotoxane terpenoids exhibit higher affinity for the housefly GABA receptor than for the rat counterpart. Our 4-subsite model satisfactorily explained the selectivity. We further showed that *seco*-prezizaane terpenoids, such as anisatin, are also antagonists, and were able to identify common pharmacophores in both groups of terpenoids that bear different skeletons.

3. ACh Receptors

Dinotefuran, a neonicotinoid, has a tetrahydrofuran ring, while other neonicotinoids have aromatic heterocyclic rings. We found that dinotefuran has a low affinity for cockroach ACh receptors labeled with [^{3}H]epibatidine and [^{3}H]α-bungarotoxin in spite of its high insecticidal activity, suggesting that another high-affinity site of action of dinotefuran might exist in the cockroach.

Little is known about what subtypes of ACh receptors are present in the insect nervous system. Novel unique ligands with a high affinity for insect receptors would be useful for characterizing insect receptor subtypes. Benzylidene anabaseines (BAs), which are derivatives of anabaseine, a worm toxin, showed nanomolar-order affinity for cockroach nicotinic ACh receptors when assayed with [^{3}H]epibatidine as a radioligand. Further modification of BAs might lead to selective insecticides.

4. Biogenic Amine Receptors

We began our studies on biogenic amine receptors, adenylate cyclase-linked, G protein-coupled receptors, by preparing phenylethylamines, phenyloxazoles, and benzaldehyde semicarbazones, which have the skeleton of octopamine. We then examined their potencies in producing cyclic AMP in homogenates of the heads of the common cutworm and silkworm. Although most of the compounds elevated cyclic AMP levels, several attenuated basal, octopamine-stimulated, and forskolin-stimulated cyclic AMP levels in silkworm head homogenates. In pharmacological studies, these compounds were shown to include selective or nonselective agonists for TA receptors. Meanwhile, we cloned a cDNA encoding a TA receptor from the silkworm, and expressed the gene in HEK-293 cells. The TA receptor attenuated intracellular cAMP production in response to TA, indicating that the TA receptor is negatively linked to adenylate cyclase. Several synthetic analogues showed a high affinity for the receptor. The TA receptor remains to be investigated as a potential target for bioregulators.

5. Concluding Remarks

Although the studies described above were mostly performed using cell membranes or native cells, recent advances in molecular biological studies have led us to define each receptor as a molecular entity. The genes encoding most neurotransmitter receptors have been isolated from *Drosophila melanogaster*. By taking advantage of the information that has been obtained, we plan to continue investigating how to target insect pest receptors with safe insecticides.