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Review

Minireview: Mode of action of meta-diamide insecticides

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ABSTRACT

Meta-diamides [3-benzamido-*N*-(4-(perfluoropropan-2-yl)phenyl)benzamides] are a distinct class of RDL GABA receptor noncompetitive antagonists showing high insecticidal activity against *Spodoptera litura.* The mode of action of the meta-diamides was demonstrated to be distinct from that of conventional noncompetitive antagonists (NCAs) such as fipronil, picrotoxin, lindane, dieldrin, and α-endosulfan. It was suggested that meta-diamides act at or near G336 in the M3 region of the *Drosophila* RDL GABA receptor. Although the site of action of the meta-diamides appears to overlap with that of macrocyclic lactones including avermectins and milbemycins, differential effects of mutations on the actions of the meta-diamides and the macrocyclic lactones were observed. Molecular modeling studies revealed that the meta-diamides may bind to an inter-subunit pocket near G336 in the *Drosophila* RDL GABA receptor better when in the closed state, which is distinct from the NCA-binding site, which is in a channel formed by M2s. In contrast, the macrocyclic lactones were suggested to bind to an inter-subunit pocket near G336 in the *Drosophila* RDL GABA receptor when in the open state. Furthermore, mechanisms underlying the high selectivity of meta-diamides are discussed. This minireview highlights the unique features of novel meta-diamide insecticides and demonstrates why meta-diamides are anticipated to become prominent insecticides that are effective against pests resistant to cyclodienes and fipronil.

1. Introduction

The cys-loop ligand-gated ion channel receptor family includes mammalian γ-aminobutyric acid type A receptors ($GABA_A$Rs), insect RDL GABA receptors, glycine receptors (GlyRs), glutamate-gated chloride channels (GluCls), nicotinic acetylcholine receptor (nAChRs), and serotonin type-3 ($5\text{-}HT_3$) receptors [1,2]. These receptors, which mediate neurotransmission, consist of five subunits. Each subunit contains a large extracellular N-terminal domain and four membrane-spanning regions designated M1–M4. Binding of ligand to an extracellular N-terminal domain induces ion permeation through a pore formed by M2s. The nAChRs and $5\text{-}HT_3$ receptors selectively permeate cations. The permeation of cations induces depolarization and causes excitation. $GABA_A$Rs, insect RDL GABA receptors, GlyRs and GluCls are selective for chloride ions. The influx of chloride ions into nerve cells induces hyperpolarization and inhibits excitatory stimuli.

The insect RDL GABA receptor is an important target for insecticides, such as the cyclodienes and fipronil. Cyclodienes and fipronil are noncompetitive antagonists (NCAs) (Fig. 1A) of the RDL GABA receptor that inhibit the GABA-induced influx of chloride ions into nerve cells, resulting in hyperexcitation of the nervous system.

Cyclodienes and lindane are first-generation insecticides that are considered to bind to the pore formed by the M2 regions of insect RDL GABA receptors. In particular, A2′and T6′ (index numbers for M2) [3] are important for their binding [4–7]. Two amino acid substitutions (A2′S and A2′G) have been reported to be associated with cyclodiene resistance in various insect species [8–15]. Furthermore, Hope et al. [16] reported that the T6′L mutation is associated with dieldrin resistance in the cattle tick *Rhipicephalus (Boophilus) microplus.*

Phenylpyrazoles, such as fipronil, are second-generation insecticides. Residues A2′ and T6′ of RDL GABA receptors are important for fipronil binding [4–7], and A2′S and A2′G mutations confer cross-resistance to fipronil; however, these mutations provide a low level of resistance [14]. It has been suggested that GABA concentration is an important factor affecting the level of fipronil resistance in fipronil-resistant *Oulema oryzae* Kuwayama carrying the A2′S mutation in their RDL GABA receptor subunit gene [15]. Fipronil has greater target site specificity for GABA receptors in insects, but not

Abbreviations: DM-RDL, *Drosophila* RDL GABA receptor subunit; EBOB, 4′-ethynyl-4-*n*-propylbicycloorthobenzoate; GABA, γ-aminobutyric acid; $GABA_AR$, GABA type A receptor; GlyR, glycine receptor; GluCl, glutamate-gated chloride channel; $5\text{-}HT_3$ receptor, serotonin type-3 receptor; meta-diamide, 3-benzamido-*N*-(4-(perfluoropropan-2-yl)phenyl)benzamide; nAChR, nicotinic acetylcholine receptor; NCA, noncompetitive antagonist; RDL, resistance to dieldrin; SL-RDL, *S. litura* RDL GABA receptor subunit.

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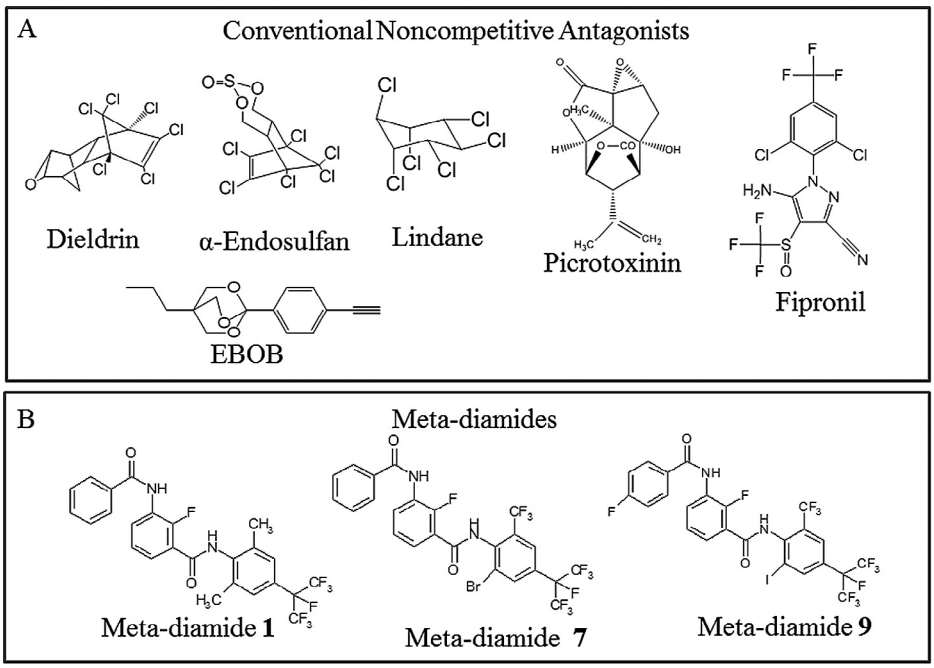

Fig. 1. Structures of conventional noncompetitive antagonists (A) and meta-diamides (B).

in mammals, compared with first-generation NCAs such as dieldrin, α-endosulfan, and lindane [17,18].

We found that the A2′N mutation is associated with fipronil resistance in the white backed planthopper (*Sogatella furcifera*) [19–21] and the small brown planthopper (*Laodelphax striatellus*) [22], which are serious rice pests. The A2′N mutation of the RDL GABA receptor was suggested to be a heterozygous mutation, and was further suggested to confer fipronil resistance [19–22].

Thus, insects have developed resistance to the conventional NCAs of the RDL GABA receptor, creating a critical need to discover and develop new insecticides effective against these insecticide-resistant pests.

Meta-diamides [3-benzamido-*N*-(4-(perfluoropropan-2-yl)phenyl)benzamides] are a distinct class of RDL GABA receptor antagonists showing high insecticidal activity against *Spodoptera litura*. This minireview assessed the characteristics of the meta-diamide insecticides that may allow them to become prominent insecticides that are effective against pests conferring resistance to the cyclodienes and fipronil.

2. Target site of the meta-diamide insecticides

The meta-diamides show insecticidal activity against the common cutworm *S. litura* [23], which is a serious pest of several crops [24,25]. The meta-diamides induce excitatory symptoms such as convulsions and paralysis. Because these symptoms are similar to those of fipronil, the effects of meta-diamides on *S. litura* RDL GABA receptor subunit (SL-RDL) homomers were examined to determine the target of meta-diamides.

Genotyping showed that the SL-RDL gene had Ser at its 2′ position, which was widely distributed among field *S. litura* larvae in Japan [23]. In this paper, the bd-type SL-RDL with Ser at its 2′ position is referred to as wild-type SL-RDLbd. *Drosophila* Mel-2 cells expressing SL-RDLbd homomers were established and a membrane potential assay was performed.

Comparison of the *S. litura* larvicidal activity of meta-diamides with their inhibitory activity against SL–RDLbd homomers revealed a linear relationship with $R^2 = 0.94$, suggesting that the RDL GABA receptor is a toxicologically relevant target of the meta-diamides [26].

According to the binding studies by Ozoe et al. [27], the N-methyl meta-diamides and their corresponding N-demethyl meta-diamides were ranked in the similar potent insecticide group against *S. litura*. N-demethyl meta-diamides inhibited the binding of [^{3}H] meta-diamide **1** to house fly head membranes with high potency. In contrast, N-methyl meta-diamides had low or little inhibitory activity. N-methyl meta-diamides may be metabolized to N-demethyl meta-diamides. Further studies are required to determine whether the metabolism of N-methyl meta-diamides is required for their larvicidal activity.

3. Effects of meta-diamide insecticides and conventional noncompetitive antagonists on wild-type and mutant SL-RDLbd homomers

Among NCAs against SL-RDLbd homomers, such as fipronil, 4′-ethynyl-4-*n*-propylbicycloorthobenzoate (EBOB), picrotoxin, lindane, dieldrin, and α-endosulfan (Fig. 1A), fipronil was the most potent antagonist, with an IC_{50} of 105 nM [26]. The inhibitory potencies of the other NCAs against SL-RDLbd homomers were low, probably because the residue at the 2′ position of SL-RDL is Ser [26]. In contrast, the IC_{50} values of meta-diamide **1, 7** and **9** (Fig. 1B) were 9.0 nM, 1.3 nM, and 3.1 nM, respectively, indicating that the inhibitory potencies of the meta-diamides against SL-RDLbd homomers are much higher than those of the NCAs [26].

Studies of S2′N, T6′V, and G319M mutations revealed a difference between the actions of NCAs and meta-diamides **1, 7**, and **9** on mutant SL-RDLbd homomers [23]. The S2′N and T6′V mutations profoundly affected the inhibitory potencies of fipronil and α-endosulfan, suggesting that fipronil and α-endosulfan acted at the 2′ and 6′ residues in M2 of the RDL GABA receptor. In contrast, a G319M mutation in the M3 region of SL-RDLbd homomers had small effects on the inhibitory activities of fipronil and α-endosulfan. The meta-diamides inhibited wild-type, T6′V, and S2′N mutant receptors with similar potencies, although the G319M mutation in SL-RDLbd abolished the inhibitory activities of the meta-diamides. Thus,

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