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Molecular Mechanism of Selective Toxicity of Neonicotinoids*

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Actions of neonicotinoids on wild-type and mutant $\alpha 7$ nicotinic receptors were investigated using voltage-clamp electrophysiology to elucidate the mechanism underlying the selectivity of neonicotinoids to insect nicotinic acetylcholine receptors. It was found that when neonicotinoids bind to the receptor, the nitro group is located close to loops D and F, which was supported by the models of the agonist binding domain of the $\alpha 7$ nicotinic receptor. Structural features of *Drosophila* D $\alpha 2$ subunit involved in interactions with imidacloprid were also studied using chimeras as well as mutant α subunits. The results suggested that the region loop B to the N-terminus, along with loop C, contributes to the selective interactions. © Pesticide Science Society of Japan

Keywords: neonicotinoid, imidacloprid, nicotinic acetylcholine receptor, selective toxicity, voltage-clamp electrophysiology, molecular modeling.

INTRODUCTION

Neonicotinoid insecticides exhibit high selective toxicity to insects over vertebrates. It has been shown that the selective toxicity of neonicotinoids is, at least in part, due to their selectivity to insect nicotinic acetylcholine receptors (nAChRs), but little is known about the molecular mechanism of selectivity. Thus the author decided to elucidate the mechanism. Despite the difficulty in expressing robust nAChRs consisting solely of insect subunits in heterologous systems such as *Xenopus laevis* oocytes, employment of the chicken $\alpha 7$ subunit (a homomer-forming, easily expressed, vertebrate subunit that does show some sensitivity to neonicotinoids), as well as a *Drosophila* D $\alpha 2$ /chicken $\beta 2$ (highly sensitive to neonicotinoids) and chicken $\alpha 4\beta 2$ (insensitive/weakly sensitive) heteromeric AChRs has led to the identification of structural features of nAChRs that contribute to the selective actions of neonicotinoids. Molecular modeling of the agonist binding site of nAChRs bound by imidacloprid has assisted in understanding the results obtained using electrophysiology combined with molecular biology.

1. ROLE OF LOOPS D AND F OF NICOTINIC ACETYLCHOLINE RECEPTORS IN THEIR INTERACTIONS WITH NEONICOTINOIDS

Most nAChRs consist of two α and three non- α subunits, but $\alpha 7$, $\alpha 8$ and $\alpha 9$ subunits form homomers when expressed in *Xenopus* oocytes. The agonist binding domain is constructed by loops A, B and C on α subunits, together with loops D, E and F on either non- α subunits, or homomer forming α subunits. Since neonicotinoids are agonists, they share binding sites with acetylcholine (ACh).

ACh always has a positive charge at the ammonium moiety, whereas imidacloprid does not have such a moiety. However, once the nitro group of imidacloprid interacts with ammonium, two nitrogens in the imidazolidine ring become positive, thereby mimicking the quaternary ammonium of ACh. It is therefore conceivable that insect nAChRs possess basic residues capable of interacting strongly with the nitro group of neonicotinoids.

With this hypothesis, the role of loop F in its interactions with neonicotinoids was examined, because this loop was proposed to interact electrostatically with ACh. The G189D and G189E mutations in loop F markedly reduced the responses to imidacloprid and nitenpyram of the $\alpha 7$ nAChRs, whereas G189N and G189Q mutations scarcely affected them, suggesting that the reduction of neonicotinoid sensitivity by the G189D and G189E mutations are attributed to an electrostatic

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repulsion between the acidic residues and the nitro group.

Since many of amino acid residues corresponding to I191 in loop F of $\alpha 7$ nAChR are tryptophan in insect non- α subunits, effects of I191W, I191F and I191Y mutations on the responses to neonicotinoids were studied. It was found that among aromatic residues, only tryptophan residue is able to enhance the interactions with neonicotinoids.

The acetylcholine binding protein (AChBP) from *Lymnaea stagnalis*, which is homologous to the agonist binding domain of the $\alpha 7$ subunit, forms a homo-pentamer. Hence, its crystal structure was thought to mimic the three-dimensional structure of the neonicotinoid binding site in the $\alpha 7$ nAChR. Q55 was found to be located close in AChBP to Y164 corresponding to G189 of the $\alpha 7$ nAChR. Thus, effects of mutations of Q79 corresponding to Q55 of AChBP on the neonicotinoid sensitivity of the $\alpha 7$ receptor were investigated. The Q79E mutation markedly reduced neonicotinoid sensitivity of the $\alpha 7$ nAChR, whereas the Q79K and Q79R mutations increased it. It was therefore postulated that Q79 in loop D is located in the vicinity of the nitro group of the insecticides in the nicotinic receptor-insecticide complex, which was supported by the actions on the mutant nAChRs of imidacloprid derivative lacking the nitro group. Interestingly, basic residues are seen in loop D of insect non- α subunits at the position corresponding to Q79 of the $\alpha 7$ nAChR, suggesting that such residues are likely to play a key role in the selectivity of neonicotinoids.

Q79 and G189 of the $\alpha 7$ nAChR were respectively replaced by basic and acidic residues to clarify relative contributions of loops D and F to interactions with imidacloprid. An increase in the responses to imidacloprid resulting from the Q79K and Q79R mutations was suppressed by a G189E mutation in loop F. Molecular modeling of the agonist binding site of the $\alpha 7$ nAChR suggests that the results may be due to electrostatic interference with the nitro group-basic residue interactions.

2. STRUCTURAL FEATURES OF α SUBUNITS CONTRIBUTING TO SELECTIVE INTERACTIONS WITH NEONICOTINOIDS

Replacement of the $\alpha 4$ subunit by the *Drosophila* D $\alpha 2$ (SAD) subunit has been shown to result in an enhancement of neonicotinoid sensitivity of the $\alpha 4\beta 2$ nAChR, indicating that the D $\alpha 2$ subunit has structural features favorable for interactions with neonicotinoids. The P242E mutation in loop C of the D $\alpha 2$ subunit reduced imidacloprid sensitivity of the D $\alpha 2$ /chicken $\beta 2$ nAChR, whereas the E219P mutation at the corresponding site of the $\alpha 4$ subunit increased that of the $\alpha 4\beta 2$ nAChR. However, the concentration-response curve for imidacloprid of the E219P mutant of the $\alpha 4\beta 2$ nAChR did not resemble that of the D $\alpha 2\beta 2$ nAChR. Thus the role of the upstream of loop B in its interactions with imidacloprid was investigated. Although replacement of the upstream of loop B of the $\alpha 4$ subunit by the corresponding region of the D $\alpha 2$ subunit was ineffective on the concentration-response curve for imidacloprid, further addition of the E219P mutation in loop C markedly shifted the curve to lower concentrations, suggesting that the upstream of loop B in the D $\alpha 2$ subunit, along with loop C, contributes to strengthening the imidacloprid-nAChR interactions.

In summary, the author has, for the first time, identified several amino acid residues playing key roles in determining the high sensitivity to imidacloprid of insect nAChRs, and modeled the nAChR-imidacloprid complexes. Also, the author has found that the region loop B to the N-terminus, in the presence of loop C, contributes to enhancing neonicotinoid sensitivity. These results provide new insights that will assist in designing safer insecticides and some of the regions highlighted may prove to be of importance in future if neonicotinoid resistance in insects emerges as a result of receptor mutations.