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Unique Neonicotinoid Binding Conformations Conferring Selective Receptor Interactions†

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ABSTRACT: Neonicotinoid agonists selectively act on the insect nicotinic acetylcholine receptor (nAChR). The molecular basis for this specificity is deciphered by comparisons of two acetylcholine binding proteins (AChBPs) with distinct pharmacological profiles that serve as structural homologues for the nAChR subtypes. *Aplysia* AChBP has high neonicotinoid sensitivity, whereas *Lymnaea* AChBP has low neonicotinoid sensitivity, pharmacologies reminiscent of insect and vertebrate nAChR subtypes, respectively. The ligand−receptor interactions for these AChBPs were established by chemical and structural neurobiology approaches. Neonicotinoids and nicotinoids bind in a single conformation with *Aplysia* AChBP, wherein the electronegative nitro or cyano pharmacophore of the neonicotinoid faces in a reversed orientation relative to the cationic nicotinoid functionality. For *Lymnaea* AChBP, the neonicotinoids have two binding conformations in this vertebrate receptor model, which are completely inverted relative to each other, whereas nicotinoids are nestled in only one conserved conformation. Therefore, the unique binding conformations of nicotinic agonists determine the selective receptor interactions.

KEYWORDS: neonicotinoid insecticides, neonicotinoid binding site interactions, neonicotinoid selectivity, nicotinic acetylcholine receptor

INTRODUCTION

The nicotinic acetylcholine receptor (nAChR) is the prototypical agonist-gated ion channel. It mediates rapid excitatory neurotransmission and is the target molecule for many toxicants, potential therapeutic agents, and insecticides (Figure 1). The nAChR subtypes in vertebrate and insect species, assembled from diverse α and non-α subunits in distinct combinations as pentameric ion channel molecules, create an incentive to design receptor subtype-selective compounds for therapeutics and crop protection with high effectiveness and maximal safety.[1,2] Preliminary attempts to understand agonist−receptor interactions involved site-directed mutagenesis or chimeragenesis, estimating the role of specific region(s) or amino acid(s) on pharmacological response. However, in this approach, attenuated or enhanced biological responses could be directly attributable to modified interacting determinant side chains in the binding site or indirectly arise from altered conformational states of the receptor. The structural biology approach of high-resolution X-ray crystallography reveals orientations of functional amino acids in ligand -bound state and conformational rearrangements of the protein upon ligand occupation.[3] Alternatively, the chemical biology strategy including incorporation of unnatural amino acids or photoaffinity labeling defines ligand−receptor recognition properties at the chemical scale in a physiologically relevant, aqueous solution environment.[2,4]

Neonicotinoid insecticides, represented by imidacloprid (IMI) and thiacloprid (THIA) (Figure 1), are agonists of the nAChR and are broadly used for crop protection, accounting for >20% of the global insecticide market.[5] Selective toxicity is critical for insecticide use, and the excellent neonicotinoid selectivity is mostly attributable to differences in target site interactions and also in part to the relative ease of penetration into the insect nervous system. Multiple strategies were necessitated in deciphering the unique aspects of neonicotinoid binding to the nAChR.[2] This paper introduces our chemical and structural biology investigations on the neonicotinoid−receptor interactions, ultimately establishing an atypical binding conformation for the selectivity.

STRUCTURE AND DIVERSITY OF NICOTINIC RECEPTORS

The vertebrate nAChR is a pentameric transmembrane structure consisting of diverse subtypes assembled from different sets of subunits expressed in skeletal muscle or electric ray (*Torpedo*) [α1, β1, γ (ε), and δ], neurons (α2-α10 and β2-β4), and sensory epithelia (α9 and α10).[6] The insect counterparts also have diverse subunits; however, the pentameric stoichiometries of the native insect nAChR subtypes have not been resolved, and some of them can be examined functionally only as recombinant hybrids consisting of various insect α subunits and a vertebrate β2 subunit.[7,8]

The functional architecture of the *Torpedo* nAChR was visualized by electron microscopy,[9] although not with adequate resolution to understand the recognition properties of the ligand binding sites. The nicotinic agonist or competitive antagonist

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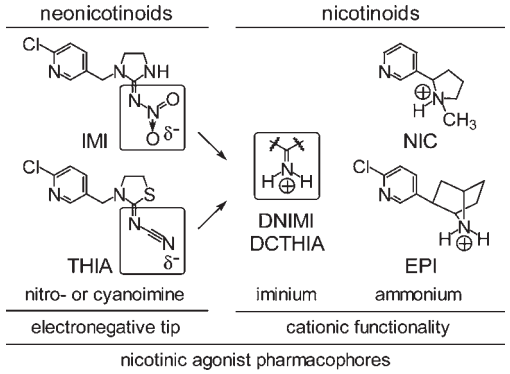

Figure 1. Neonicotinoid and nicotinoid chemotypes of nicotinic agonists with an electronegative pharmacophore and cationic functionality, respectively.

Table 1. Binding Affinity of Nicotinic Ligands to the nAChR and AChBP Subtypes

	K_i (nM)			
	nAChR		AChBP	
nicotinic ligand	insect[a]	vertebrate[b]	*Aplysia*[c]	*Lymnaea*[d]
Neonicotinoids with an Electronegative Pharmacophore				
IMI-$CHNO_2$ analogue[e]	0.12	60	1.7	80
THIA	1.2	240	3.9	220
IMI	3.0	970	19	970
acetamiprid[f]	7.2	680	32	1200
Nicotinoids with a Cationic Functionality				
(±)-EPI	290	0.01	1.0	0.3
DCTHIA	130	1.2	0.6	16
DNIMI	1000	2.2	15	18
(−)-NIC	2700	1.9	30	100

[a] Native *Drosophila* brain nAChR assayed with [^{3}H]IMI binding. [b] Recombinant chick α4β2 nAChR determined by [^{3}H]NIC binding. [c] Y55W mutant evaluated with [^{3}H]acetamiprid. [d] Assayed with [^{3}H]EPI binding. [e] 1-(6-Chloropyridin-3-ylmethyl)-2-nitromethyleneimidazolidine. [f] (*E*)-*N*^{1}-(6-Chloropyridin-3-ylmethyl)-*N*^{2}-cyano-*N*^{1}-methylacetamidine.

binding pocket is localized at interfacial regions between subunits and consists of several discontinuous loops. Understanding drug−nAChR interactions was greatly facilitated by the discovery and crystallization of soluble acetylcholine binding proteins (AChBPs) from the freshwater snail *Lymnaea stagnalis* and the saltwater mollusk *Aplysia californica* as structural surrogates for the extracellular ligand-binding domain of the nAChR.[10−12]

NICOTINIC AGONIST CHEMOTYPE AND RECEPTOR SUBTYPE SELECTIVITY

Neonicotinoids and nicotinoids, such as the naturally occurring alkaloids nicotine (NIC) and epibatidine (EPI), are similar in providing a common pyridin-3-yl moiety, whereas they are crucially different in their pharmacophores (Figure 1). The neonicotinoid is coplanar between the guanidine or amidine plane and the nitro or cyano substituent, yielding electronic conjugation to facilitate partial negative charge (δ^-) flow toward the tip.[13,14] This unique molecular system therefore serves as the neonicotinoid pharmacophore. On the other hand, the nicotinoid chemotype has a basic nitrogen atom being predominantly protonated (ammonium cation) at physiological pH, that is, cationic functionality. Interestingly, the desnitro or descyano derivatives (DNIMI or DCTHIA) are protonated (iminium cation) under physiological pH as with nicotinoids.[15]

Neonicotinoids are selective for insect nAChRs and nicotinoids for vertebrate receptors (Table 1). This opposite selectivity profile is practically replicated by two AChBP subtypes. *Aplysia* AChBP is highly sensitive to neonicotinoids and nicotinoids. In marked contrast, the *Lymnaea* AChBP subtype has lower affinity for neonicotinoids than nicotinoids. Thus, the *Aplysia* AChBP serves as a plausible structural surrogate for interactions of both neonicotinoids with the insect nAChR and nicotinoids with the vertebrate receptor. Furthermore, the *Lymnaea* AChBP can be a homologue for the vertebrate nAChR.[16,17]

NEONICOTINOID AND NICOTINOID BINDING SITE INTERACTIONS

Neonicotinoid and nicotinoid binding site interactions in chemical or atomic resolution have been defined by both photoaffinity labeling with mass spectrometry technology and X-ray crystallography investigations using AChBP subtypes,[16−20] thereby establishing the two structural models for interfacial agonist-binding domains of nAChR subtypes of aphid (*Myzus persicae*) α2β1 (for neonicotinoids) and chick α4β2 (for nicotinoids) (Figure 2).

The IMI chloropyridinyl chlorine atom can have favorable van der Waals interactions with the backbone of loop E amino acids such as Asn and Leu. The pyridine nitrogen atom forms a water bridge to the backbone NH of Ile and the carbonyl oxygen of Asn (loop E) (Figure 2). Interestingly, the electronically conjugated guanidine plane primarily π-stacks with the loop C Tyr aromatic side chain and also interacts via stacking or hydrophobic interactions with the loop B Trp indole moiety. The nitro oxygen or cyano nitrogen tip undergoes hydrogen bonding with the loop C Cys and/or Val backbone. Relative to nicotinoid DNIMI, as with IMI, the chloropyridinyl moiety identically interacts with the corresponding loop E amino acids of the chick α4β2 receptor. Importantly, the nicotinoid cationic functionality (iminium or ammonium head) critically contacts the carbonyl oxygen of loop B Trp via hydrogen bonding, and this interaction is stabilized by cation−π contacts with the loop B Trp and other aromatic residues of loops A, C, and D. Accordingly, the nicotinoid cationic functionality is nestled in a reverse direction compared with the neonicotinoid electronegative pharmacophore (Figure 2).

MOLECULAR RECOGNITION CONFERRING SELECTIVE NEONICOTINOID INTERACTION

The structural determinants of nAChR subtype selectivity have been studied for a family of peptide antagonists with a binding region extending over a large interfacial surface to embrace a unique moiety of the antagonist.[12,21−23] However, the molecular mechanism of selectivity for small agonist molecules is less well resolved because most of the key amino acids in the nAChR binding pocket are conserved in all of the receptor subtypes and species. The amino acids forming the binding pockets are structurally or functionally consistent not only in the diverse nAChR subtypes but also in the AChBPs, yet there is considerable neonicotinoid selectivity. Chimera hybrid receptor consisting of insect α subunit and vertebrate β2 subunit, wherein either insect loop D, E, or F sequence was

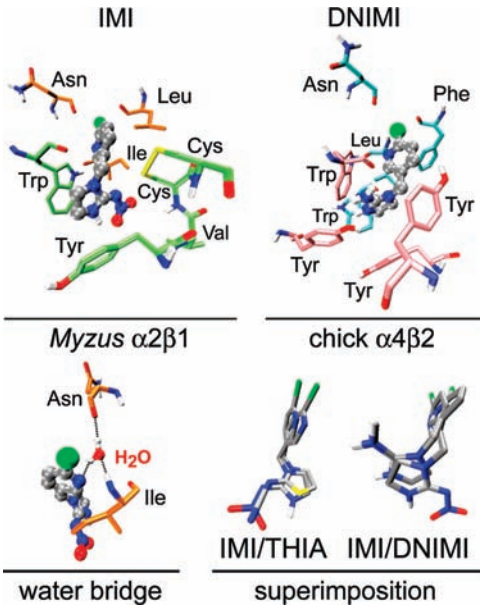

Figure 2. Structural models for binding site interactions of IMI and DNIMI with the $\alpha-\beta$ subunit interfacial agonist-binding pocket of nicotinic receptors based on chemical and structural biology investigations. Representative neonicotinoid IMI and nicotinoid DNIMI are nestled in the aphid (*Myzus*) $\alpha2\beta1$ and chick $\alpha4\beta2$ interfaces, respectively (upper). Amino acids in green or pink are from aphid α2 or chick α4 subunit, and those in orange or cyan are from aphid $\beta1$ or chick $\beta2$ subunit, respectively. A water molecule near the pyridine nitrogen atom, captured in AChBP-IMI or AChBP-THIA crystal structure (Protein Data Bank code 3C79 or 3C84, respectively),[19] is superimposed onto this IMI-bound structure (lower left). Consistently, a water or solvent bridge is also observed in the AChBP crystals liganded with nicotinoids NIC and EPI.[11,12] Binding conformations of IMI, THIA, and DNIMI as observed in the agonist-binding pocket are compared (lower right). IMI and THIA are nicely superimposable, whereas IMI and DNIMI [or THIA and DCTHIA (not shown)] pharmacophores are reversed relative to each other. DNIMI- and EPI-bound conformations are suitably overlaid (not shown).

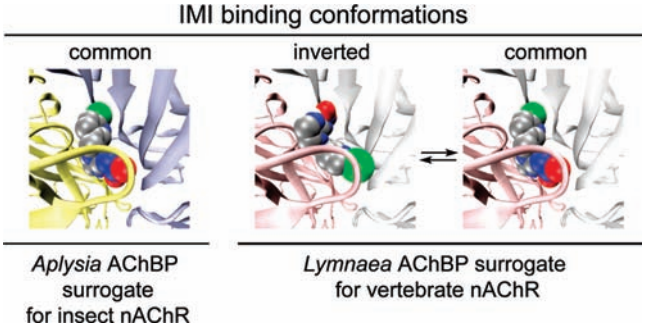

Figure 3. Comparative binding conformations of IMI embraced by *Aplysia* AChBP as an insect nAChR homologue (left) and by *Lymnaea* AChBP as a surrogate for the vertebrate nAChR (middle and right). IMI binds in two conformations designated "common" and "inverted".

inserted, attenuates IMI-elicited agonist responses. However, the influence of the chimeragenesis is modest and of similar magnitude among the three chimera hybrid receptors.[24,25] This result clearly points out a distinct limitation for the mutagenesis or chimeragenesis approach to pinpoint the specific region or amino acid(s) for neonicotinoid selectivity.

Alternatively, photoaffinity labeling with *Lymnaea* AChBP subtype as the vertebrate receptor homologue led to structurally defining the mechanism of neonicotinoid selectivity[17] (Figure 3). *Lymnaea* AChBP accommodates the neonicotinoids in two distinct bound conformations. One binding orientation is completely inverted compared with the common conformation (which is the one observed in the *Aplysia* subtype). Therefore, a blend of two very disparate binding conformations at the *Lymnaea* AChBP and vertebrate nAChR coincides with the inferior affinity of neonicotinoids at these sites, possibly contributing to the poor binding constant, which reflects a weighted average of a multiplicity of binding orientations. Only a single tight binding conformation at the *Aplysia* AChBP as in the insect nAChR model confers high neonicotinoid sensitivity. In nicotinoids, a single binding orientation is conserved for all AChBP and nAChR subtypes.[17] The final binding constant represents a combination of multiple individual constants unique to different conformations. The same agonist molecule can also assume different binding directions at other Cys-loop receptors depending upon the nature and position of the aromatic amino acids.[4,26,27]

CONCLUDING REMARKS

High neonicotinoid affinity and selectivity toward the insect nAChR are ultimately attributable to the fundamentally different binding site interactions, which have been defined in chemical and atomic scale using mollusk AChBP structural homologues. Neonicotinoids with the nitroguanidine or cyanoamidine pharmacophore are embraced by a reversed position compared with the nicotinoid cationic functionality. A single dominant binding orientation presumably causes the high affinity for neonicotinoids at the insect nAChR and different positioning for nicotinoids at the vertebrate nAChR. However, the inferior potency of neonicotinoids at the vertebrate nAChR model is associated with multiple binding conformations in the agonist-binding pocket. These findings, in molecular recognition conferring agonist potency and selectivity, facilitated illustrative studies on nAChR structure-guided insecticide design.[28−31] Accordingly, the nicotinic receptor target warrants continuing research to discover novel nicotinic insecticides with unique biological properties, high effectiveness, and maximal safety.

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ABBREVIATIONS USED

AChBP, acetylcholine binding protein; DCTHIA, descyano-THIA; DNIMI, desnitro-IMI; EPI, epibatidine; IMI, imidacloprid; NIC, nicotine; nAChR, nicotinic acetylcholine receptor; THIA, thiacloprid.

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