

NEONICOTINOID INSECTICIDE RECEPTORS

Motohiro Tomizawa¹ from the University of California at Berkeley discusses current understanding of structure and diversity of insect nicotinic acetylcholine receptors, and its importance in the development of new insecticides

Introduction

Crop protection and veterinary pest control have changed greatly with the recent introduction of neonicotinoid (or chloronicotinyl) insecticides represented by imidacloprid, the only major new class of chemical insecticides of the last three decades. They are increasingly utilized throughout the world (billion-dollar-a-year market), and seven neonicotinoid insecticides are commercialized or nearly on the market at present and are expected to become fourth major insecticide group following the organophosphates, methylcarbamates and pyrethroids (Kagabu, 1997; Yamamoto and Casida, 1999). Neonicotinoids selectively act on the insect nervous system as agonists of the nicotinic acetylcholine receptor (nAChR). They exhibit not only high affinity to the receptor but also suitable physicochemical properties such as nonionizability and moderate water solubility, thereby providing an incentive to explore the structure and diversity of insect nAChRs. The nAChR is a neurotransmitter-regulated ion channel complex that is responsible for rapid synaptic transmission (Figure 1). In central nervous system of insect, the nAChR plays a major role and is an important target for insecticide action. Therefore the insect nAChRs have been investigated with pharmacological, biochemical, molecular biological and immunohistochemical approaches but are still poorly understood compared to vertebrate nAChRs (for reviews, Yamamoto and Casida, 1999; Tomizawa, 2000). This article reviews the current research status for the insect nAChR describing the functional architecture and diversity, and also describes the contribution of neonicotinoids.

An outline of the vertebrate nAChR

The vertebrate nAChR consists of diverse subtypes assembled as five subunits in combinations from nine α (α 1-9), four β (β 1-4), δ , γ and ϵ subunits (Figure 1). The skeletal muscle or electric ray (*Torpedo*) subtype is made up of two

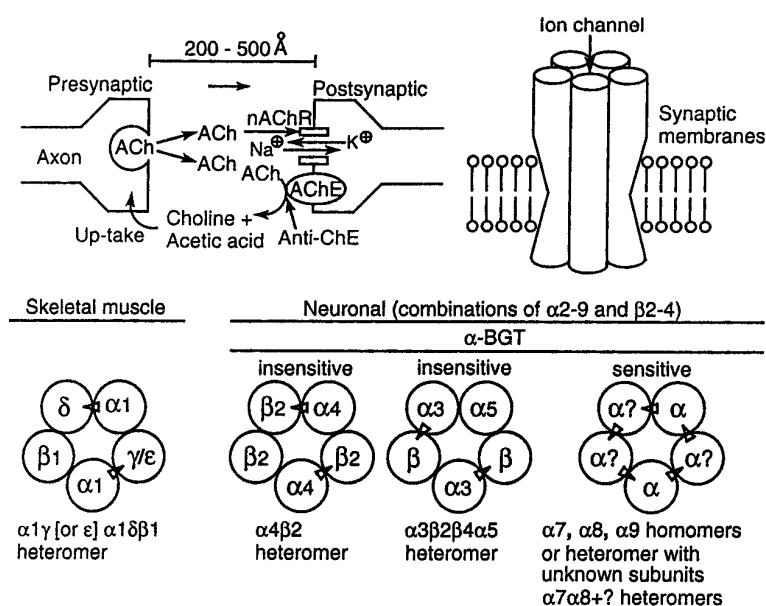


Figure 1. Functional architecture and diversity of the vertebrate nicotinic acetylcholine receptor (nAChR). Top left panel represents neurotransmission through cholinergic synapse. The right is an image of the membrane-associated nAChR. The bottom illustration displays the typical vertebrate nAChR subtypes (cross-section images, seen from top of receptor). The receptor is assembled from five subunits, and different subunit combinations make the receptor subtype. The α 8 subunit exists only in the avian brain, ganglia and retina. Wedges designate binding site regions for cholinergic agents.

α 1 subunits and one each of β 1, δ , γ (or ϵ in adult muscle) subunits and is best understood relative to the ligand-binding site environment. Neuronal nAChR subtypes expressed in vertebrate brain and ganglia are assembled in combinations of α 2-9 and β 2-4 and are pharmacologically classified into two branches based on sensitivity to the antagonist α -bungarotoxin (a snake toxin, α -BGT). The α -BGT-insensitive branch is made up of subtypes with combinations of α 2-6 and β 2-4 subunits. Of these, the α 3 β X α 5 (X=2 and/or 4) subtype is mainly found in ganglia and the α 4 β 2 subtype is the most abundant subtype in brain. The α 7-9 subunits are involved in the α -BGT sensitive subtypes in brain and ganglia, etc., and the amount of α 7-containing receptor is comparable to that of the α 4 β 2 subtype in brain. The specific subunit combinations confer differences in the pharmacological profile among the receptor subtypes, since the drug-binding site is localized at the interface region between subunits (Figure 1). The mammalian nAChR is a

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target for several potential therapeutic agents for neuropathic disorders, cognitive enhancement and analgesia, leading to a recent interest in the possibility of subtype-selective agents at this receptor (for reviews, Lindstrom, 1997; Decker and Meyer, 1999; Cordero-Erausquin *et al.*, 2000). In addition, existence of the multiple receptor subtypes should also be considered in the insecticide safety.

Structure and diversity of insect nAChRs

Molecular biology approaches

In the fruit fly, *Drosophila melanogaster*, three genes encoding the ligand-binding α -type ($D\alpha 1$, $D\alpha 2$ and $D\alpha 3$) subunits and two for the structural β -type subunits have been identified based on conserved sequence. However, expression of homomeric receptors with either the $D\alpha 1$ (ALS) or $D\alpha 2$ (SAD) alone does not generate a functional receptor and the *Drosophila* β -type (ARD and SBD) subunits do not contribute to functional receptor expression. Coexpression of the *Drosophila* α - and β -type subunits in various combinations does not produce any electrophysiological or biochemical response. At present, the functional receptor with ion channel property and/or radioligand binding activity can be generated only when any of the three α -type subunits is coexpressed with the vertebrate (chick or rat) β -type subunit. These results strongly suggest the importance of the β - or non- α -type subunit and the heterooligomeric status of the native *Drosophila* nAChR with possible involvement of unidentified subunit(s) (Bertrand *et al.*, 1994; Lansdell and Millar, 2000; for review, Tomizawa, 2000). Interestingly, the antibodies raised against $D\alpha 1$ and $D\alpha 2$ subunits can visualize specific regions in the *Drosophila* central nervous system and their distribution patterns are quite similar. Furthermore, it has been demonstrated that both of the above two subunits are functionally coassembled with the chick $\beta 2$ subunit in *Xenopus* oocyte within a single receptor complex, suggesting the possibility that the insect nAChR consists of three subunits (Schulz *et al.*, 2000). Recently, the understanding of the nAChR of the peach-potato aphid *Myzus persicae* has rapidly increased. Five genes encoding the α -type ($Mp\alpha 1-5$) and one for the β -type ($Mp\beta 1$) subunit have been cloned. As with *Drosophila*, functional architecture of the native *Myzus* nAChR is not yet available (Huang *et al.*, 1999; Huang *et al.*, 2000). Also, nAChR α - and β -type subunit genes have been cloned in the migratory locust, *Locusta migratoria*. Each one of α -type subunit gene has been found in the desert locust *Schistocerca gregaria* or the tobacco hornworm *Manduca sexta* (for review, Tomizawa, 2000).

Protein biochemistry approaches: Neonicotinoids as probes for insect nAChRs

Neonicotinoid insecticides act at the insect nAChR, providing a new approach to isolating insect nAChRs and to accessing the insecticide-binding site (Figure 2). [^3H]imidacloprid has been developed as an insect receptor-selective radioligand with extremely high affinity and high specificity (Liu and Casida, 1993). This radioligand has made a great contribution to the study on insect nAChR as well as evaluation of compound potency. The second successful

approach is a neonicotinoid-agarose matrix for affinity isolation of the native nAChRs from *Drosophila* and house fly (*Musca domestica*) head preparations. This approach demonstrated for the first time the possibility that the native insect nAChR may consist of multiple subunits (Tomizawa *et al.*, 1996). Photoaffinity labeling has been used to characterize the environment of the insecticide-binding site in the receptor. In principle, the photoaffinity radioligand is specifically bound to the site, and then a covalent bond is formed by photochemical reaction between the photoreactive group in the ligand and the critical amino acid residue(s) in a specific site. On this basis, we have developed an [^{125}I]azido-neonicotinoid photoaffinity probe and suggested for the first time that the insecticide-binding subunit(s) is possibly localized at interface area between two subunits in the *Drosophila* nAChR (Tomizawa and Casida, 1997). Furthermore, in the Casida group at Berkeley, extensive synthetic efforts have been targeted at developing a candidate probe with a photoreactive group at a more suitable position in the neonicotinoid molecule.

Existence of insect nAChR subtypes?

In vertebrate nAChRs, differential subunit combinations make the receptor subtypes. In the insect nAChR, multiple subunit genes have been cloned, implying the existence of subtypes that may display different pharmacological properties. Interestingly, hybrid nAChRs consisting of the *Drosophila* $D\alpha 1$ /chick $\beta 2$ and $D\alpha 3$ /chick $\beta 2$ is sensitive to α -BGT, but the *Drosophila* $D\alpha 2$ /chick $\beta 2$ hybrid receptor is insensitive to α -BGT. On the other hand, imidacloprid binds to all of the above three hybrid receptors with high affinity (Bertrand *et al.*, 1994; Schulz *et al.*, 1998; Lansdell and Millar, 2000). Similarly, two types of nAChRs based on the sensitivity to α -BGT exist in the dorsal unpaired median neuron of the American cockroach (*Periplaneta americana*), and both are sensitive to imidacloprid (Buckingham *et al.*, 1997). In *Myzus*, the hybrid receptors made up of either *Myzus* $Mp\alpha 1$, 2 or 3 with rat $\beta 2$ subunits show differences in imidacloprid sensitivity: i.e. imidacloprid binds to $Mp\alpha 2$ /rat $\beta 2$ and $Mp\alpha 3$ /rat $\beta 2$ receptors, while not to $Mp\alpha 1$ /rat $\beta 2$ receptor (Huang *et al.*, 1999). At present, coexpression of the insect α subunits with the vertebrate β

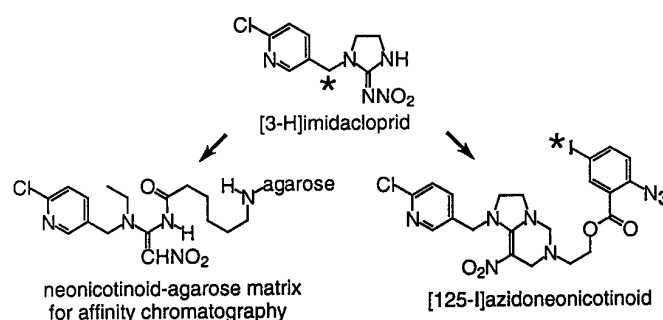


Figure 2. The neonicotinoid insecticide has provided the opportunity to devise probes for exploring the functional architecture and diversity of the insect nicotinic acetylcholine receptor (nAChR).

subunit constitutes the best available model, although these hybrid receptors may not accurately reflect the insect nAChRs. This strongly suggests that additional insect nAChR subunits remain to be identified.

Summary

This article summarizes current status in insect nAChR research. It is impossible to depict a clear model of the insect nAChR relative to structure and diversity. In vertebrate nAChRs, the subtype-directed drug exploration is a recent trend for the development of the agent with specificity and reduced adverse side-effects (Decker and Meyer, 1999). Hence identification and characterization of the insect nAChR subtypes are also becoming an attractive research field and may open up an era of subtype-selective or species-selective insecticides. This approach might lead to specific insecticide with a minimum application dose and a preferential safety factor for non-target species. The neonicotinoid related compounds (metabolites and analogs) act on multiple mammalian nAChR subtypes with selectivity conferred by only minor structural modification (Tomizawa and Casida, 1999). Therefore, subtype or subunit selectivity might also be expected in the insect nAChR subunit level. The challenge with neonicotinoid insecticide probes is to make contributions to understanding the functional architecture and diversity of the insect nAChRs. More importantly, neonicotinoid insecticides provide an excellent example of selective toxicity mechanism between insect and vertebrate. Thus, clarification of the structural differences between insect pest and vertebrate nAChR in their insecticide-binding sites will be very helpful for the development of more effective and safer insecticides.

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References

Bertrand, D.; Ballivet, M.; Gomez, M.; Bertrand, S.; Phannavong, B.; Gundelfinger, E. D. (1994) Physiological properties of neuronal nicotinic acetylcholine receptors reconstituted from the vertebrate $\beta 2$ subunit and *Drosophila* α subunits. *European Journal of Neuroscience* **6**, 869–875.

Buckingham, S. D.; Laped, B.; Corronc, H. L.; Grolleau, F.; Sattelle, D. B. (1997) Imidacloprid actions on insect neuronal acetylcholine receptors. *Journal of Experimental Biology* **200**, 2685–2692.

Cordero-Erausquin, M.; Marubio, L. M.; Klink, R.; Changeux, J.-P. (2000) Nicotinic receptor function: New perspectives from

knockout mice. *Trends in Pharmacological Science* **21**, 211–217.

Decker, M. W.; Meyer, M. D. (1999) Therapeutic potential of neuronal nicotinic acetylcholine receptor agonists as novel analgesics. *Biochemical Pharmacology* **58**, 917–923.

Huang, Y.; Williamson, M. S.; Devonshire, A. L.; Windass, J. D.; Lansdell, S. J.; Millar, N. S. (1999) Molecular characterization and imidacloprid selectivity of nicotinic acetylcholine receptor subunits from the peach-potato aphid *Myzus persicae*. *Journal of Neurochemistry* **73**, 380–389.

Huang, Y.; Williamson, M. S.; Devonshire, A. L.; Windass, J. D.; Lansdell, S. J.; Millar, N. S. (2000) Cloning, heterologous expression and co-assembly of M $\rho\beta 1$, a nicotinic acetylcholine receptor subunit from the aphid *Myzus persicae*. *Neuroscience Letters* **284**, 116–120.

Kagabu, S. (1997) Chloronicotiny insecticides-discovery, application and future perspective. *Reviews in Toxicology* **1**, 75–129.

Lansdell, S. J.; Millar, N. S. (2000) The influence of nicotinic receptor subunit composition upon agonist, α -bungarotoxin and insecticide (imidacloprid) binding affinity. *Neuropharmacology* **39**, 671–679.

Lindstrom, J. (1997) Nicotinic acetylcholine receptors in health and disease. *Molecular Neurobiology* **15**, 193–222.

Liu, M.-Y.; Casida, J. E. (1993) High affinity binding of [3 H]imidacloprid in the insect acetylcholine receptor. *Pesticide Biochemistry and Physiology* **46**, 40–46.

Schulz, R.; Sawruk, E.; Mlhardt, C.; Bertrand, S.; Baumann, A.; Phannavong, B.; Betz, H.; Bertrand, D.; Gundelfinger, E. D.; Schmitt, B. (1998) D $\alpha 3$, a new functional α subunit of nicotinic acetylcholine receptors from *Drosophila*. *Journal of Neurochemistry* **71**, 853–862.

Schulz, R.; Bertrand, S.; Chamaon, K.; Smalla, K.-H.; Gundelfinger, E. D.; Bertrand, D. (2000) Neuronal nicotinic acetylcholine receptors from *Drosophila*: Two different types of α subunits coassemble within the same receptor complex. *Journal of Neurochemistry* **74**, 2537–2546.

Tomizawa, M. (2000) Insect nicotinic acetylcholine receptors: Mode of action of insecticide and functional architecture of the receptor. *Japanese Journal of Applied Entomology and Zoology* **44**, 1–15.

Tomizawa, M.; Casida, J. E. (1997) [125 I]Azidonicotinoid photoaffinity labeling of insecticide-binding subunit of *Drosophila* nicotinic acetylcholine receptor. *Neuroscience Letters* **237**, 61–64.

Tomizawa, M.; Casida, J. E. (1999) Minor structural changes in nicotinic insecticides confer differential subtype selectivity for mammalian nicotinic acetylcholine receptors. *British Journal of Pharmacology* **127**, 115–122.

Tomizawa, M.; Latli, B.; Casida, J. E. (1996) Novel neonicotinoid-agarose affinity column for *Drosophila* and *Musca* nicotinic acetylcholine receptors. *Journal of Neurochemistry* **67**, 1669–1676.

Yamamoto, I.; Casida, J. E. (Eds.) (1999) Nicotinoid insecticides and the nicotinic acetylcholine receptor. Springer-Verlag, Tokyo, 300 pp.

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