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Pharmacological profiles of recombinant and native insect nicotinic acetylcholine receptors

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Abstract

Nicotinic acetylcholine receptors (nAChRs) are targets for insect-selective neonicotinoid insecticides exemplified by imidacloprid (IMI) and mammalian-selective nicotinoids including nicotine and epibatidine (EPI). Despite their importance, insect nAChRs are poorly understood compared with their vertebrate counterparts. This study characterizes the [^{3}H]IMI, [^{3}H]EPI, and [^{3}H]α-bungarotoxin (α-BGT) binding sites in hybrid nAChRs consisting of *Drosophila melanogaster* (fruit fly) or *Myzus persicae* (peach–potato aphid) α2 coassembled with rat β2 subunits (Dα2/Rβ2 and Mpα2/Rβ2) and compares them with native insect and vertebrate α4β2 nAChRs. [^{3}H]IMI and [^{3}H]EPI bind to Dα2/Rβ2 and Mpα2/Rβ2 hybrids but [^{3}H]α-BGT does not. In native *Drosophila* receptors, [^{3}H]EPI has a single high-affinity binding site that is independent from that for [^{3}H]IMI and, interestingly, overlaps the [^{3}H]α-BGT site. In the Mpα2/Rβ2 hybrid, [^{3}H]IMI and [^{3}H]EPI bind to the same site and have similar pharmacological profiles. On considering both neonicotinoids and nicotinoids, the Dα2/Rβ2 and Mpα2/Rβ2 receptors display intermediate pharmacological profiles between those of native insect and vertebrate α4β2 receptors, limiting the use of these hybrid receptors for predictive toxicology. These findings are consistent with the agonist binding site being located at the nAChR subunit interface and indicate that both α and β subunits influence the pharmacological properties of insect nAChRs.
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Keywords: *Drosophila*; Epibatidine; Hybrid nicotinic receptor of *Drosophila* α2 or *Myzus* α2 and rat β2 subunits; Imidacloprid; Neonicotinoids

1. Introduction

The nicotinic acetylcholine receptor (nAChR) is an agonist-gated ion channel complex for rapid excitatory neurotransmission. It is widely distributed in the insect central nervous system and constitutes a major target for insecticide action (Tomizawa and Casida, 2003). The functional architecture and diversity of insect nAChRs are superficially understood in contrast to their vertebrate counterparts. Genes encoding the ligand-binding α and structural β subunits have been cloned in several insect species. For instance in the fruit fly (*Drosophila melanogaster*), seven α (Dα1–7) and three β (Dβ1–3) subunit genes are cloned. Also in the peach–potato aphid (*Myzus persicae*), a major target pest for neonicotinoid insecticides, there are six identified genes for five α (Mpα1–5) and a β (Mpβ1) subunits (Gundelfinger and Schulz, 2000; Tomizawa and Casida, 2001; Grauso et al., 2002; Millar, 2003; Lansdell and Millar, 2004). The first four *Drosophila* α subunit genes (Dα1–4) and three β subunit genes (Dβ1–3), on expression in *Xenopus* oocytes, human embryonic kidney 293 cells or *Drosophila* S2 cells, singly or in various combinations, never produce any electrophysiological response or radioligand binding (Tomizawa and Casida,

Abbreviations: α-BGT, α-bungarotoxin; B_{max}, maximal binding capacity; CLO, clothianidin; D*α2*/R*β2*, hybrid receptor consisting of *Drosophila* α2 and rat β2 subunits; DCTHIA, descyano-THIA; DNIMI, desnitro-IMI; EPI, (±)-epibatidine; IMI, imidacloprid; K_D, dissociation constant; K_i, inhibition constant; Mpα2/Rβ2, hybrid receptor consisting of *Myzus* α2 and rat β2 subunits; nAChR, nicotinic acetylcholine receptor; NIC, (−)-nicotine; n_H, Hill coefficient; PBS, phosphate-buffered saline; THIA, thiacloprid

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2001; Millar, 2003). Dα6 and Dα7 genes can be expressed as chimera receptors containing the C-terminal domain of the serotonin-3A receptor to give [^{125}I]α-bungarotoxin (α-BGT) and [^{3}H]methyllycaconitine binding activities (Lansdell and Millar, 2004). *Myzus* Mpα1 and Mpα2 subunits form functional homomeric receptors expressed in *Xenopus* oocytes (Sgard et al., 1998) but no radioligand binding is detected with any of the four α subunits (Mpα1–4) alone or in combinations with Mpβ1 expressed in *Drosophila* S2 cells (Huang et al., 1999, 2000). However, functional ion channel property or radioligand binding activity is observed when any one of the four *Drosophila* α (Dα1–4) or three *Myzus* α (Mpα1–3) subunits is coexpressed in a hybrid receptor with a chick or rat β2 subunit (Bertrand et al., 1994; Schulz et al., 1998; Sgard et al., 1998; Huang et al., 1999; Lansdell and Millar, 2000a, b).

Neonicotinoids (Fig. 1) are the only major new class of insecticides developed in the past three decades with worldwide annual sales currently accounting for 15% of the total insecticide market (Nauen et al., 2001; Matsuda et al., 2001; Kagabu, 2003; Tomizawa and Casida, 2003, 2005). The excellent selective toxicity of neonicotinoids between insects and vertebrates is mainly conferred by the differential sensitivity of the insect versus vertebrate nAChRs (Tomizawa and Casida, 2003, 2005). As an example, the neonicotinoid radioligand [^{3}H]imidacloprid ([^{3}H]IMI) serves as an excellent probe for insect but not vertebrate nAChRs (Liu and Casida, 1993; Tomizawa and Casida, 2003). [^{3}H]Epibatidine ([^{3}H]EPI) and [^{125}I]- or [^{3}H]α-BGT are important probes for characterizing the

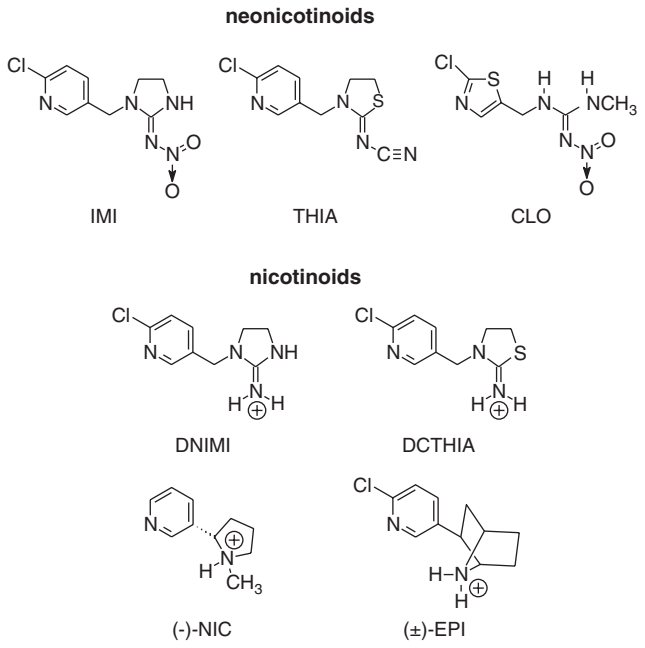

Fig. 1. Structures of three neonicotinoid insecticides and four nicotinoids (including two metabolites of neonicotinoids). Abbreviations: IMI, imidacloprid; THIA, thiacloprid; CLO, clothianidin; DNIMI, desnitro-IMI; DCTHIA, descyano-THIA; (−)-NIC, (−)-nicotine; (±)-EPI, (±)-epibatidine.

vertebrate α4β2 and α7 nAChR subtypes, respectively (Anand et al., 1993; Houghtling et al., 1995). In native insect nAChRs, the [^{3}H]IMI binding site in *Drosophila* is distinct from that for [^{3}H]α-BGT (Zhang et al., 2004). Specific [^{3}H]EPI binding has been detected in some insects such as the American cockroach (*Periplaneta americana*) but not in others such as the housefly (*Musca domestica*) (Orr et al., 1997; Nauen et al., 2001).

Pharmacological profiles of the recombinant hybrid insect α/vertebrate β nAChRs are poorly defined and the binding sites are not established for identified subunits versus native receptors. Dα2 and Mpα2 are considered to be the main neonicotinoid-binding components based on protein biochemistry and immunology studies (Chamaon et al., 2002; Tomizawa and Casida, 1997; Tomizawa et al., 1996, 2001a) and sensitivity to IMI for the hybrid receptors (Lansdell and Millar, 2000a; Huang et al., 1999; Ihara et al., 2003). In this report, we characterize the [^{3}H]IMI and [^{3}H]EPI binding sites in two recombinant hybrid nAChRs co-assembled by either Dα2 or Mpα2 with rat β2 subunits (Dα2/Rβ2 and Mpα2/Rβ2 receptors) and the pharmacological properties are compared with those of the native *Drosophila* and *Myzus* nAChRs and the vertebrate α4β2 subtype.

2. Methods

2.1. Chemicals

Sources for the compounds were: [^{3}H]EPI (53 Ci/mmol) and [^{3}H]α-BGT (37 Ci/mmol) from Amersham Biosciences (Piscataway, NJ, USA); [^{3}H]IMI (32 Ci/mmol) from Syngenta Crop Protection (Basel, Switzerland); (−)-nicotine hydrogen tartrate (NIC) and α-BGT from Sigma (St. Louis, MO, USA); (±)-EPI hydrochloride from TOCRIS (St. Louis, MO, USA). The neonicotinoids and nicotinoids were available from previous studies in the Berkeley laboratory (Tomizawa et al., 2000).

2.2. Cell culture

Schneider's *Drosophila* S2 cells stably expressing the Dα2/Rβ2 or Mpα2/Rβ2 hybrid nAChR were described earlier (Lansdell et al., 1997; Huang et al., 1999; Lansdell and Millar, 2000a). They were grown at 25 °C in Sheilds and Sang M3 medium (Sigma) supplemented with 12.5% heat inactivated (at 56 °C for 30 min) fetal bovine serum, 100 units/ml penicillin, and 0.1 mg/ml streptomycin (Gibco Life Technologies, Grand Island, NY, USA). Expression of nAChR subunit cDNAs from the metallothionein promoter of pRmHa3 was induced by addition of $CuSO_4$ (0.6 mM final concentration) for 24 h prior to binding assay. Mouse fibroblast M10 cells stably transfected with chick α4β2 subunits were donated by Professor Jon M. Lindstrom (University of Pennsylvania Medical School, Philadelphia, PA, USA) and cultured as reported (Whiting et al., 1991).

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