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Agonist actions of neonicotinoids on nicotinic acetylcholine receptors expressed by cockroach neurons

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Abstract

The agonist actions of seven commercial neonicotinoid insecticides and nicotine were studied on nicotinic acetylcholine receptors (nAChRs) expressed by neurons isolated from the three thoracic ganglia of the American cockroach, *Periplaneta americana.* Single electrode voltage clamp recording was used to measure agonist-induced inward currents. Acetylcholine, nicotine and all neonicotinoids tested, except thiamethoxam, caused inward currents which were blocked reversibly by methyllycaconitine, a nAChR antagonist. Based on maximum inward currents, neonicotinoids could be divided into two subgroups: (1) those with a heterocyclic ring in their electronegative pharmacophore moiety (i.e. nicotine, imidacloprid and thiacloprid) were relatively weak partial agonists causing only 20–25% of the maximum ACh current and (2) open chain compounds (i.e. acetamiprid, dinotefuran, nitenpyram, and clothiandin) which were much more effective agonists producing 60–100% of the maximum ACh current. These compounds also elicited different symptoms of poisoning in American cockroaches with excitatory responses evident for the low efficacy agonists but depressive and paralytic responses predominating for the most efficacious agonists. No correlation was observed between agonist affinity and efficacy on these nAChRs. Thiamethoxam, even at 100 μM, failed to cause an inward current and showed no competitive interaction with other neonicotinoids on nAChRs, indicating that it is not a direct-acting agonist or antagonist. Despite the probable presence of multiple subtypes of nAChRs on cockroach neurons, competition studies between neonicotinoids did not reveal evidence that separate binding sites exist for the tested compounds. The size of inward currents induced by co-application of neonicotinoid pairs at equal concentration (100 μM) were predominantly determined by the one with higher binding affinity as indicated by EC_{50} values, rather than by the one with higher binding efficacy as indicated by maximal current (I_{max}). Agonist efficacy, but not affinity, was positively correlated with insecticidal activity. These findings indicate that: (1) agonist affinity and efficacy vary independently with neonicotinoid structure; (2) high agonist efficacy is dependent on the presence of an acyclic electronegative pharmacophore group; (3) agonist efficacy is a significant factor in the insecticidal activity of neonicotinoids to cockroaches; (4) lower efficacy compounds cause excitatory symptoms (Type A), while high efficacy compounds cause depressive/paralytic symptoms (Type B).

Keywords: Single electrode voltage clamping recording; Neonicotinoids; Nicotinic acetylcholine receptors (nAChRs); Imidacloprid; Thiacloprid; Acetamiprid; Dinotefuran; Clothianidin; Nitenpyram; Thiamethoxam; Nicotine; American cockroach; German cockroach

1. Introduction

Neonicotinoids, such as imidacloprid (IMI), are increasingly deployed for pest management and have become an important class of insecticides (Kagabu, 1997; Tomizawa and Casida, 2003). Up to now, six additional neonicotinoids have been marketed since the introduction of IMI in 1991 (Elbert et al., 1990; Thyssen and Machemer, 1999). These neonicotinoids are acetamiprid (ACT) (Takahashi et al., 1992), nitenpyram (NIT) (Minamida et al., 1993), thiacloprid (THI) (Elbert et al., 2000), thiamethoxam (TMX) (Senn et al., 1998), clothianidin (CTD) (Ohkawara et al., 2002), and dinotefuran (DTF) (Kodaka et al., 1998; Wakita et al., 2003). Their structures (Fig. 1) are characterized by having an aromatic heterocyclic chloropyridinylmethyl (CPM), chlorothiazolylmethyl (CTM), or tetrahyrdofurylmethyl (TFM) moiety coupled with either a cyclic or an open-chain electronegative pharmacophore group containing an *N*-nitroimine, 2-nitromethylene or *N*-cyanoimine

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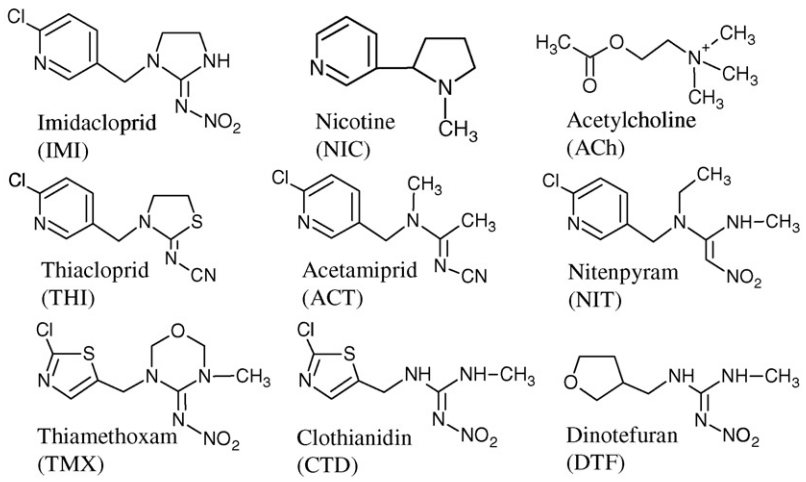

Fig. 1. Structures of nicotine, acetylcholine and seven neonicotinoids. In the neonicotinoids, a chloropyridinylmethyl (CPM) group is coupled with a cyclic *N*-nitroimine moiety in IMI and an *N*-cyanoimine moiety in THI, with an acyclic *N*-cyanoimine moiety in ACT and with a 2-nitromethylene moiety in NIT. A chlorothiazolylmethyl (CTM) group is coupled with a cyclic *N*-nitroimine moiety in TMX and an acyclic *N*-nitroimine moiety in CTD. A tetrahydrofurylmethyl (TFM) group is coupled with an acyclic *N*-nitroimine moiety in DTF. Nicotine and acetylcholine are two naturally occurring nAChR agonists. The three-letter abbreviations for these compounds are used in all figures and text.

moiety. Electrophysiological studies and radioligand binding assays have established that neonicotinoid insecticides share a common mode of action in selectively targeting insect nicotinic acetylcholine receptors (nAChRs), acting as agonists or antagonists (Buckingham et al., 1997; Matsuda et al., 2005; Nauen et al., 2001a; Tomizawa and Casida, 2005; Zwart et al., 1994).

The described agonist actions of neonicotinoids on nAChRs include partial, full and super activities. The application of IMI activates nAChRs and elicits inward currents on cultured Kenyon cells of the honeybee, *Apis mellifera,* and on *Drosophila*-chicken SADβ2 and ALSβ2 hybrid nAChRs expressed in *Xenopus laevis* oocytes, acting as a partial agonist that elicits 24–55% of the maximum ACh-induced currents (Déglise et al., 2002; Ihara et al., 2003, 2004; Matsuda et al., 1998). However, on honeybee antennal lobe neurons, IMI can act either as a full agonist or as a partial agonist, suggesting that IMI has different actions on different subtypes of insect nAChRs (Nauen et al., 2001b). Other neonicotinoids, such as CTD or DTF, with an acyclic moiety corresponding to the imidazolidine moiety of IMI, induce either a greater maximum response than ACh or the same response as ACh on SADβ2 hybrid nAChRs (Ihara et al., 2004; Kagabu et al., 2002). Thus, they are referred to as super or full agonists, respectively. This diversity of agonist activity of neonicotinoids appears to be related both to their structure and to the subtypes of nAChRs expressed by the target cells, but no comparison of all the commercial neonicotinoids has been made on a single insect neuronal preparation.

It is still unclear whether neonicotinoids agonist efficacy (I_{max}) is related to insecticidal activity as efficacy does not necessarily parallel binding affinity as indicated by EC_{50} value (concentration producing half maximal response) (Ihara et al., 2004). However, Nishiwaki et al. (2003) demonstrated that the EC_{50} for neonicotinoids on the Dα2β2 hybrid nAChRs expressed in *Xenopus* oocytes, have a higher positive correlation with the insecticidal activity against houseflies ($r = 0.893$) than the I_{max} values, suggesting that affinity is a more important determinant of insecticidal activity than efficacy. Nevertheless, a contribution of agonist efficacy to insecticidal activity cannot be ruled out, since neonicotinoids with higher efficacy depolarize neurons to a greater degree than those with lower efficacy. Clothianidin and DTF show high insecticidal activity and induce greater maximum responses than IMI on Dα2β2 hybrid nAChRs (Ihara et al., 2004; Kagabu et al., 2002), but their EC_{50} values on Dα2β2 hybrid nAChRs measured by two electrode voltage clamp electrophysiology are lower than those of IMI. In order to elucidate in more detail the contribution of agonist action to the insecticidal activity of neonicotinoids, it is essential to measure their agonist actions on nAChRs expressed by native neurons from insects.

In this paper, we studied the agonist actions and interactions of the seven commercial neonicotinoids on native nAChRs expressed by thoracic neurons from the American cockroach, *Periplaneta americana*. Our data provide an overall picture of the structural factors that contribute to the agonist actions on cockroach neuronal nAChRs. We also examined the potential relationship between their agonist actions and insecticidal activities.

2. Materials and methods

2.1. Chemicals

All neonicotinoids except nitenpyram were obtained as analytical standards (≥99% purity) from the USEPA. Nitenpyram (analytical standard), nicotine hydrogen tartrate (98%), acetylcholine chloride (99%), methyllycaconitine citrate, atropine (99%) and piperonyl butoxide (90%) were obtained from Sigma–Aldrich (St. Louis, MO).