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# Exploring the pharmacological properties of insect nicotinic acetylcholine receptors

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**Insect nicotinic acetylcholine (nACh) receptors are molecular targets of insecticides such as neonicotinoids that are used to control disease-carrying insects and agricultural pests. To date, several insect nACh receptor subunits have been identified, indicating different nACh receptor subtypes and pharmacological profiles. Because of the difficulty in expressing functional insect nACh receptors in heterologous systems, new research tools are needed. Studies on insects resistant to the insecticide imidacloprid and on laboratory-generated hybrid and chimaeric nACh receptors *in vitro* have provided information about the molecular basis of receptor diversity, neonicotinoid resistance and selectivity. Additionally, recent results indicate that the sensitivity of insect nACh receptors to imidacloprid can be modulated by intracellular phosphorylation mechanisms, which offers a new approach to studying insect nACh receptor pharmacology.**

## Molecular diversity of insect nicotinic acetylcholine receptor subunits

Insect nicotinic acetylcholine (nACh) receptor subunits, like vertebrate nACh receptor subunits, consist of a large N-terminal extracellular domain involved in agonist binding, followed by three transmembrane regions (TM1–TM3, with TM2 lining the channel), a large intracellular loop, a fourth transmembrane domain (TM4) and a C-terminal extracellular region (Figure 1). The presence of two vicinal cysteine residues, equivalent to Cys192 and Cys193 in the electric organ (*Torpedo marmorata*) α1 subunit, which are known to be involved in ACh binding, defines nACh receptor α subunits in insects, vertebrates and all other species whereas non-α subunits (β, γ, δ or ε) lack this motif. From comparison of the insect and vertebrate nACh receptor subunit sequences, it is evident that six insect monophyletic groups diverged from a common ancestor distinct from the one that led to the vertebrate subunits (e.g. α1, α2, α3, α4, α8 and β1 subunits). One such group includes all the β subunits except for the *Drosophila* Dβ2 and Dβ3 subunits. Additional subunits share a common ancestor with the vertebrate α7 and α8 subunits, which characteristically form α-bungarotoxin (α-Bgt; see Glossary)-sensitive nACh receptor subtypes, thereby defining a monophyletic group of α7-like subunits [1,2] (Figure 2). Consequently, as previously shown [3,4], subunit combinations determine the distinct pharmacological properties of insect nACh receptors and the sensitivity to neonicotinoid insecticides of insect nACh receptors compared with their vertebrate counterparts. The pharmacological properties of insect nACh receptors have been investigated using several different approaches, including vertebrate–insect hybrid receptors and mutation of residues believed to be involved in ligand binding. In the present review, we discuss emerging data on the pharmacological properties of insect nACh receptors and offer new tools for their study.

## Evidence for different nACh receptor subtypes

The subunit composition of native insect nACh receptors remains unclear, largely because the heterologous expression of functional insect nACh receptors has proved difficult. Nevertheless, behavioural studies using different nicotinic agonists and antagonists [5,6] or *Drosophila* Dα7 mutants [7] have established that the insect central

### Glossary

**α-Bungarotoxin (α-Bgt)**: toxin from snake venom. α-Bgt binding is considered to represent the distribution of α7-subunit-containing nACh receptors.
**DEG**: degeneration of certain neurons. In *Caenorhabditis elegans*, there are 42 different nACh receptor subunits, including the *deg-3* group. *des-2* is another gene in this group.
**DES**: degeneration suppressor. Mutations in the gene encoding this protein suppress the degeneration caused by *deg-3*.
***Drosophila* Dα7 mutant**: excisions of *P* elements in the Dα7 subunits lead to several alleles.
**nACh receptor subtype**: a specific combination of identical (homomeric) or different (heteromeric) subunits that forms a pentameric nACh receptor.

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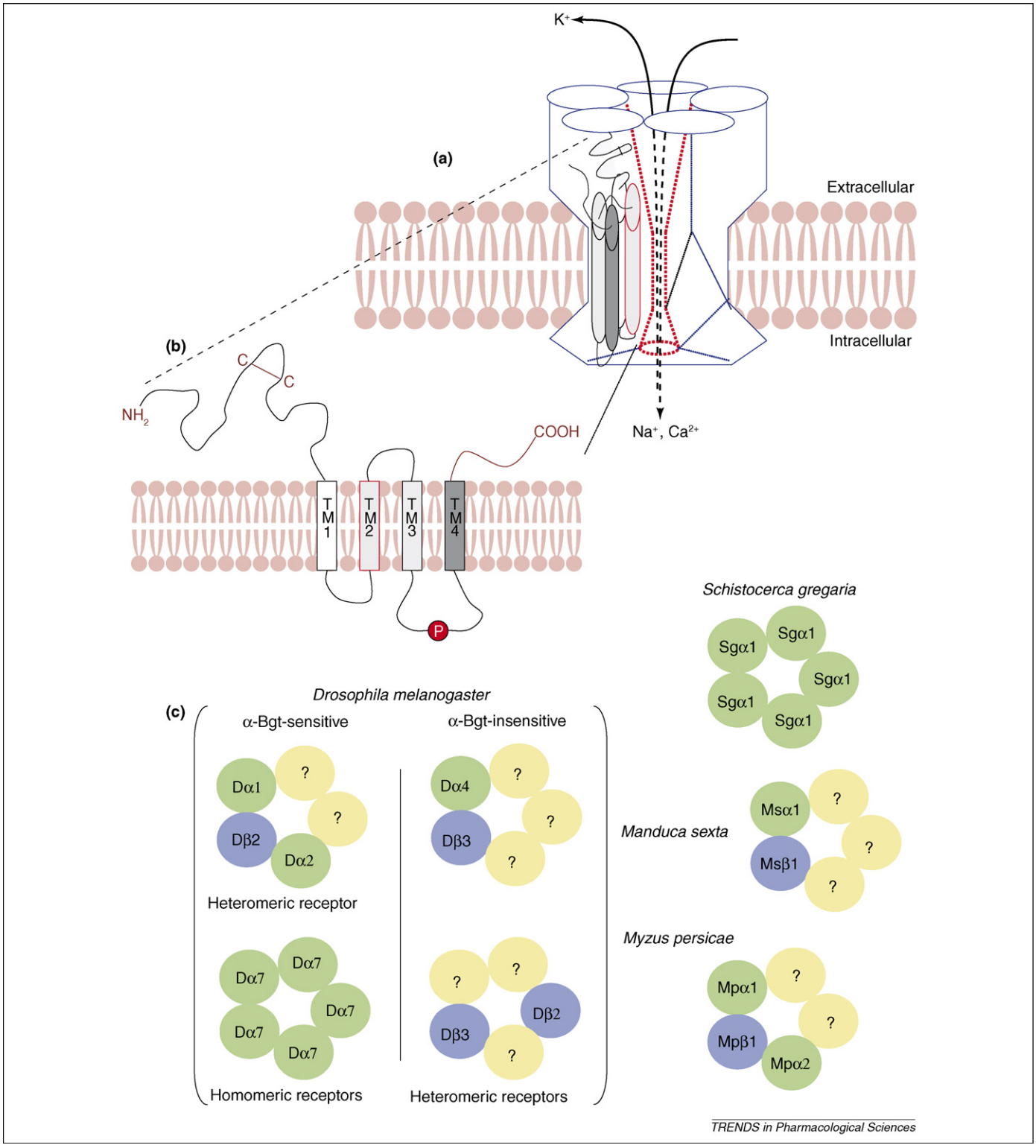


**Figure 1**. Insect nACh receptor subtypes. **(a)** nACh receptors are pentameric macromolecules composed of five identical subunits (homomeric receptors) or different subunits (heteromeric receptors) arranged around a central pore, which is selective to $Na^+$, $K^+$ and $Ca^{2+}$. **(b)** Each subunit comprises four transmembrane domains, TM1–TM4, with TM2 lining the ion channel. Between transmembrane domains TM3 and TM4 is a potential phosphorylation site. **(c)** An example of potential insect α-bungarotoxin-sensitive and -insensitive nACh receptors from *Drosophila melanogaster*, and other nACh receptor subtypes from *Schistocerca gregaria, Manduca sexta* and *Myzus persicae.* Question marks denote unknown subunits.

nervous system expresses different nACh receptor subtypes. This finding is reinforced by electrophysiological studies showing that, as in vertebrates, insect neurons can express different nACh receptor subtypes (Table 1). Pharmacological profiling shows that there exist at least two pharmacologically distinct classes of nACh receptors, α-Bgt-sensitive and α-Bgt-insensitive. As in vertebrates, insect α7-like subunits (e.g. Dα5, Dα6 and Dα7 subunits of *Drosophila*) are potential candidates to form α-Bgt-sensitive receptors [7,8]. However, Dα1, Dα2, Dα3, Dβ1 and Dβ2 subunits can be copurified by α-Bgt affinity chromatography, indicating that: (i) nACh receptors comprising

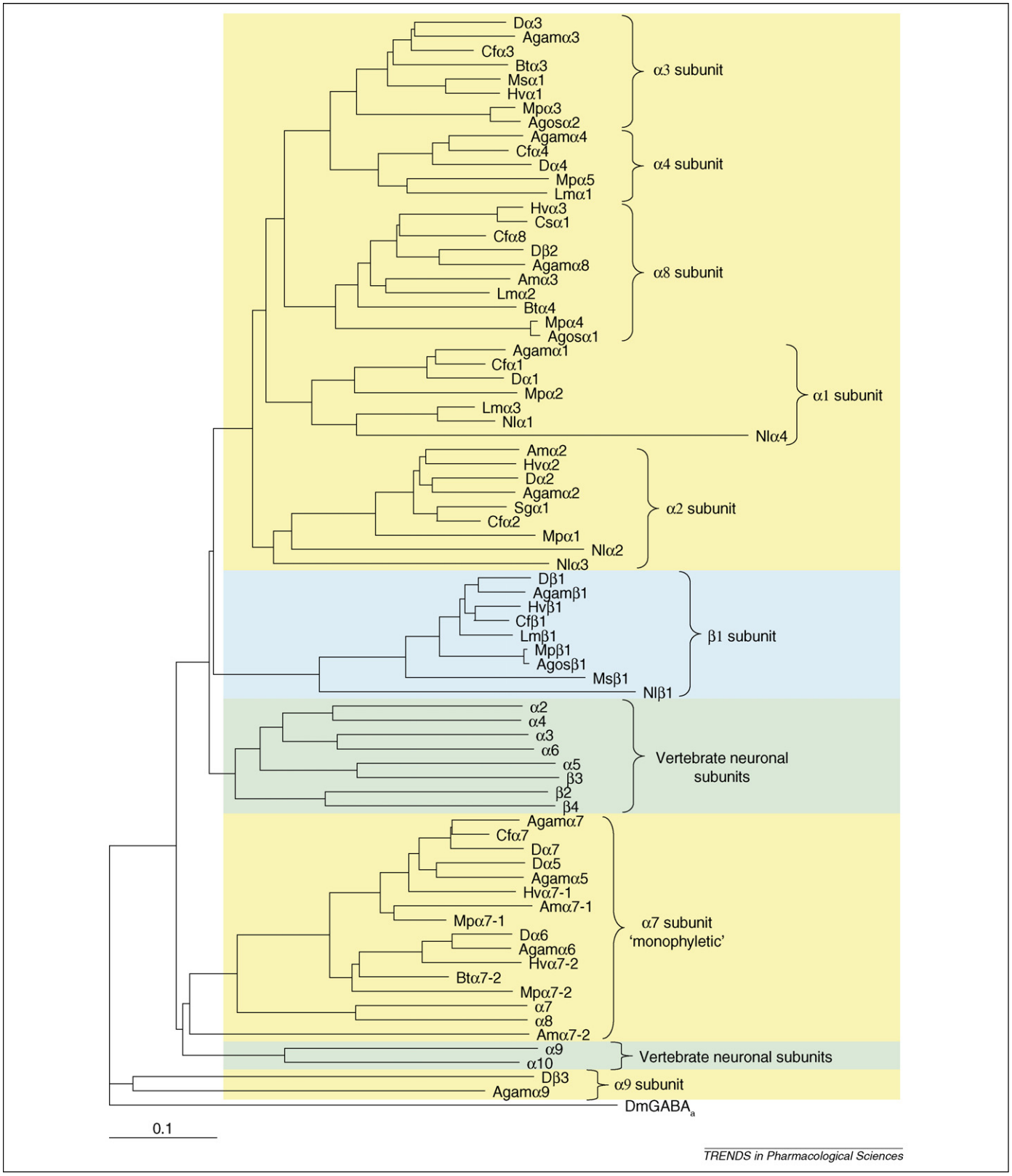


**Figure 2**. Phylogenetic tree showing relationship of insect nACh receptor and vertebrate neuronal subunits. A *Drosophila* $GABA_A$ subunit, $DmGABA_A$ (GenBank accession number AAA28556) was used as an outgroup. *Apis mellifera*: Amα2 (AY540846), Amα3 (AF514804), Amα7-1 (AY500239), Amα7-2 (AY569781). *Drosophila melanogaster*: Dα1 (X07194), Dα2 (X52274), Dα3 (Y15593), Dα4 (AJ272159), Dα5 (AAM13390), Dα6 (AF321445), Dα7 (CAD86936), Dβ1 (X04016), Dβ2 (X55676), Dβ3 (AJ318761). *Heliothis virescens*: Hvα1 (CAA04056), Hvα2 (AF096678), Hvα3 (AAD09809), Hvα7-1 (AF143846), Hvα7-2 (AF173847), Hvβ1 (AF096880). *Locusta migratoria*: Lmα1 (AJ000390), Lmα2 (AJ000391), Lmα3 (AJ000392), Lmβ (AJ000393). *Manduca sexta*: Msα1 (Y09795), Msβ1 (AJ007397). *Myzus persicae*: Mpα1 (X81887), Mpα2 (X81888), Mpα3 (AJ236786), Mpα4 (AJ236787), Mpα7-1 (CAI54102), Mpα7-2 (CAI54103), Mpβ (AJ251838). *Schistocerca gregaria*: Sgα1 (X55439). *Nilaparvata lugens*: Nlα1 (AAQ75737), Nlα2 (AAQ75731), Nlα3 (AAQ75739), Nlα4 (AAQ75738), Nlβ1 (AAQ75742). *Chilo suppressalis*: Csα1 (AAL40742). *Aphis gossypii*: Agosα1 (AAM94383), Agosα2 (AAM94382), Agosβ1 (AAM94383). *Ctenocephalides felis*: Cfα1 (ABB42999), Cfα2 (ABB43000), Cfα3 (ABB43001), Cfα4 (ABB43003), Cfα7 (ABB43004), Cfα8 (ABB43002), Cfβ1 (ABB43005). *Bemisia tabaci*: Btα3 (CAI54098), Btα4 (CAI54099), Btα7-2 (CAI54100). *Anopheles gambiae*: Agamα1 (AAU12503), Agamα2 (AAU12504), Agamα3 (AAU12505), Agamα4 (AAU12506), Agamα5 (AAU12508), Agamα6 (AAU12509), Agamα7 (AAU12511), Agamα8 (AAU12512), Agamα9 (AAU12513), Agamβ1 (AAU12514). *Homo sapiens*: α2 (U62431), α3

**Table 1. Pharmacological profiles of insect native nACh receptors[a]**

| Species | Identified cell types | Ligands tested | | Refs |
|---|---|---|---|---|
| | | Agonists | Antagonists | |
| *Periplaneta americana* | DUM neurons | ACh, Nic, Imi (nACh receptor 1) | D-TC (inhibition of nACh receptor 1), Mec, α-conotoxin (inhibition of nACh receptor 2) | [41,42] |
| | Df motor neuron | ACh, Nic, carbachol | 5-HT, dopamine and octopamine reduce the amplitude of nACh responses | [49] |
| | Thoracic ganglia neurons | ACh, clothianidin, Epi, Imi (inhibition of the desensitized component, nAChD) | α-Bgt (inhibition of both nAChD and the nondesensitized component, nAChN), MLA (inhibition of nAChN) | [40] |
| *Apis mellifera* | Kenyon cells | ACh and carbamylcholine (full agonists), Nic, Epi, Cyt and Imi (partial agonists) | α-Bgt, DHβE and MLA (full agonists), Mec, D-TC and Hex (weak blockers) | [50,51] |
| | Antennal lobe neurons | ACh; Epi and Imi (partial agonists); olefin and 5-OH-Imi | α-Bgt (partial antagonist)[b], MLA, DHβE (full antagonists) | [52,53] |
| *Drosophila melanogaster* | Kenyon cells | The properties of the acetylcholine synaptic currents were assessed from analysis of EPSC | α-Bgt blocks the ACh-induced current | [54] |
| | Ventral nerve cord neurons | ACh | D-TC (reversible inhibition) | [55] |
| | Clock neurons | ACh, Nic | Mec (incomplete blocking with lower concentration) | [56] |
| *Manduca sexta* | Abdominal ganglia neurons | ACh, Nic | D-TC and Mec (full antagonist on nACh receptor containing MARA1 subunit) | [57,58] |
| *Heliothis virescens* | Ventral nerve cord neurons | *N*-desmethyl thiamethoxam, clothianidin, Imi | nd | [59] |
| *Locusta migratoria* | Thoracic ganglia neurons | ACh, physostigmine (partial competitive agonist) | D-TC, bicuculline, hydrastine, gabazine (partial antagonist) | [60] |

[a]Abbreviations: Df, fast coxal depressor; DHβE, dihydro-β-erythroidine; D-TC, D-tubocurarine; Epi, epibatidine; EPSC, excitatory postsynaptic current; Hex, hexamethonium; Imi, imidacloprid; Mec, mecamylamine; MLA, methyllycaconitine; nd, not determined; Nic, nicotine.
[b]Only one concentration tested.

these subunits can bind to α-Bgt; and (ii) they can form heteromeric α-Bgt-sensitive receptors [9,10]. Thus, it seems that insect native α-Bgt-sensitive receptors can be either heteromeric or homomeric. Indeed, Dα5, Dα6 or Dα7 can form homomeric [7] or heteromeric receptors containing additional α or β subunits [8,11].

## nACh receptor-associated proteins: possible roles in function and assembly

Molecular cloning followed by functional expression in either *Xenopus laevis* oocytes or cultured mammalian cell lines has enabled studies of the physiology and pharmacology of vertebrate nACh receptors of defined subunit composition, which can reflect native nACh receptor subtypes [12–15]. Unfortunately, these approaches have failed in insect nACh receptor subunit expression studies, except for the *Schistocerca* nACh receptor Sgα1 [16,17]. In vertebrates, crucial control steps for functional expression include transcriptional regulation [18], subunit folding and assembly [19,20], transport [21,22], clustering and surface stability [23]. However, the reasons for the poor heterologous surface expression of insect nACh receptor subunits are not well understood. Thus, it is essential to identify cellular factors that influence the expression of functional insect nACh receptors to enable robust heterologous expression and, therefore, provide a new research tool. For example, it has been shown that coexpression of the protein encoded by the resistance to inhibitors of cholinesterase gene (*ric-3*) in *Xenopus laevis* oocytes enhances (i) the functional expression of the *Caenorhabditis elegans* nACh receptor DEG-3/DES-2 [19] and (ii) the formation of functional α7 receptors expressed in mammalian cells [24,25]. Interestingly, *ric-3* belongs to a conserved gene family found in both vertebrates and invertebrates [19,20]. However, because the role of *ric-3* depends on the identity of the coexpressed receptors [25], it is not possible to say, *a priori*, if coexpression of this protein would lead to successful heterologous expression of insect nACh receptors [20,24]. Other factors, such as transcriptional elements located at the 5′-noncoding region of the α9 subunits [18] and intracellular factors (e.g. chaperone, scaffolding or adaptor proteins, such as the 14-3-3 protein family [21]), can enhance the expression levels of nACh receptors. The lack of such important nACh receptor-associated proteins could explain in part the failure to express robust functional insect nACh receptor subunits in cell lines commonly used for expression.

## Selectivity of neonicotinoids for insect nACh receptors

The neonicotinoid insecticides, such as imidacloprid, show sensitivity for both native and recombinant insect nACh receptors, attributable in part to the imidazolidine ring [26,27]. Chemical modification of this imidazolidine ring can lead to greater affinity, as is the case with clothianidin [27], for example. In studies of *Drosophila*–chicken Dα2–β2 and Dα1–β2 hybrid receptors expressed in *Xenopus laevis* oocytes, the neonicotinoid ligands tested [e.g. des-nitro-imidacloprid, nitempyram and the nitro-

(M37981), α4 (L35901), α5 (M83712), α6 (U62435), α7 (X70297), α9 (CAB65091), α10 (AJ278118), β2 (X53179), β3 (X67513), β4 (X68275). *Gallus gallus*: α8 (X52296). For the bee subunits, Amα2 is equivalent to Amelα2, Amα3 is equivalent to Amelα8, Amα7-1 is equivalent to Amelα7, and Amα7-2 is equivalent to Amelα5 [61].

methylene analogue of imidacloprid (CH-IMI)] activate the Dα2–β2 receptors. By contrast, imidacloprid and CH-IMI are ineffective in activating the Dα1–β2 receptors [28]. These results reveal that the α subunit contributes to the selectivity of imidacloprid for insect nACh receptors and that specific residues in the Dα2 subunit could explain enhancement of neonicotinoid affinity [28]. Mutation of the proline at position 242 to glutamic acid (P242E) in the *Drosophila* Dα2 subunit (Figure 3) induces a shift of the half effective concentration ($EC_{50}$) and $I_{max}$ values for

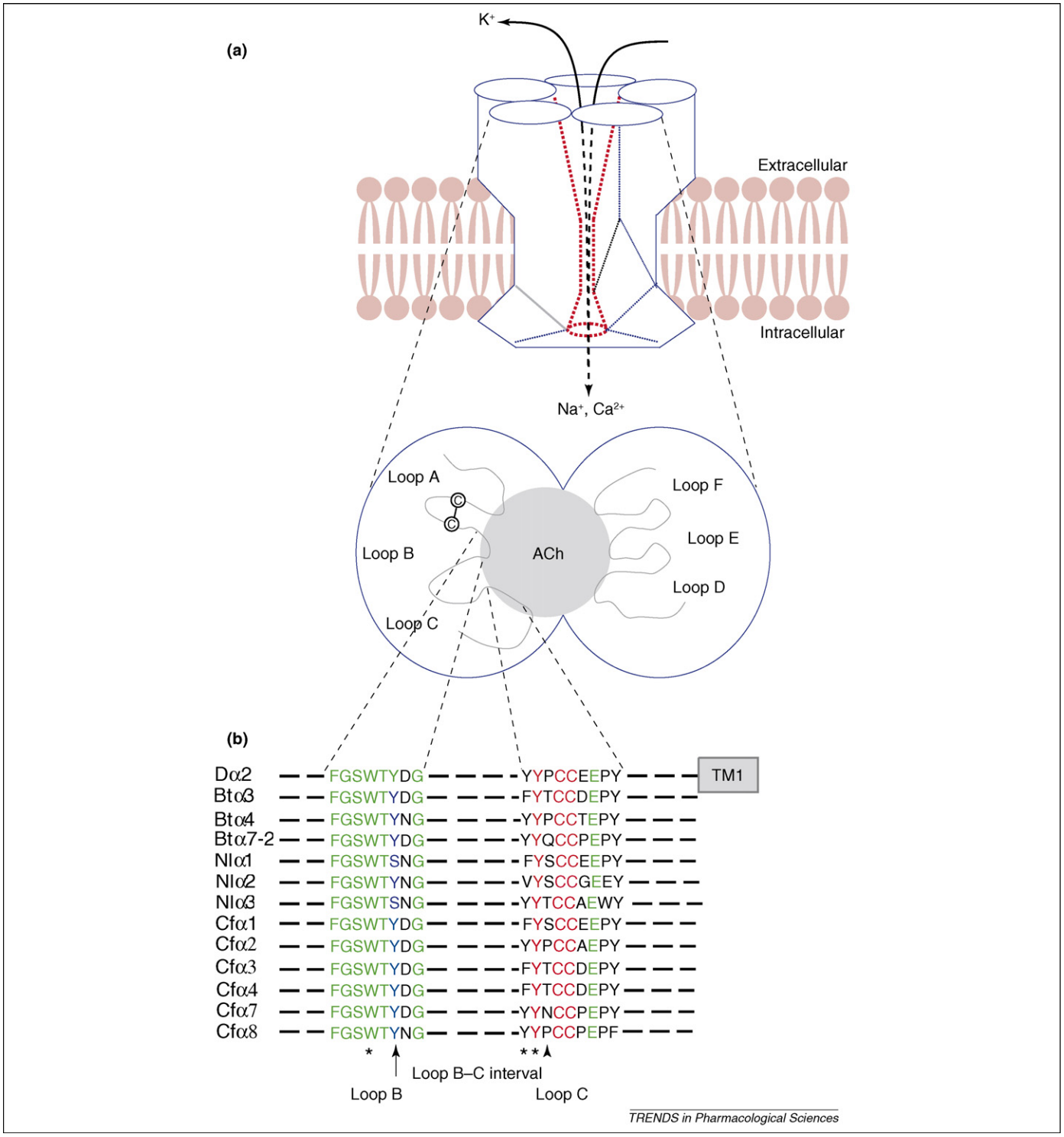


**Figure 3**. The nACh receptor and ACh-binding domain. **(a)** At the interface between two subunits, the location of the six loops (A–F) believed to be important in forming the binding site for both agonist and antagonist are indicated in relation to the ACh-binding domain. Three loops (A, B and C) are contributed by the α subunit interface and three loops (D, E and F) by the non-α interface in heteromeric receptors. **(b)** Loops B and C of insect subunits showing resistance to imidacloprid attributable to a specific subunit. The serine residue in loop B, conferring resistance to imidacloprid, is indicated by the arrow. The arrowhead indicates the position of proline in the YXCC motif. Insertion of this proline in the Dα2–α4 chimaeric receptor results in a marked displacement to the left of the concentration–response curve for imidacloprid. The two adjacent cysteine residues characteristic of loop C in the α subunit are indicated in red. Conserved residues in loops B and C are indicated in green. The conserved tryptophan residue in loop B and two tyrosine residues in loop C are indicated by asterisks. Note that these tyrosine residues are not conserved in *Bemisia tabaci* Btα3, *Nilaparvata lugens* Nlα1 and *Ctenocephalides felis* Cfα1, Cfα3 and Cfα4.

## Box 1. Intracellular pathways regulating insect nACh receptor function

Both activation (+) and inhibition (−) of insect nACh receptors are regulated by phosphorylation-dependent mechanisms (Table I, Figure I). The activity of the 'target proteins' is stimulated by elevation of the concentration of intracellular 'second messengers', which is modulated by different enzyme activities or variation of the intracellular calcium concentration ($[Ca^{2+}]_i$), or both.

Insect nACh receptor function can be mediated by cAMP pathways. At relatively low internal concentrations, cAMP increases the nicotine-induced current but at greater concentrations it reduces current amplitude. This bell-shaped dose-dependent effect occurs through the cAMP–PKA cascade by the activation of adenylyl cyclase (AC), which maintains the cAMP level necessary to modulate nACh receptor responses. Additionally, AC activity can be regulated by intracellular $Ca^{2+}$ acting through the calcium-receptor protein CaM, indicating that $Ca^{2+}$/calmodulin-dependent protein kinase II (CaMKII) can affect nACh receptor function directly through $[Ca^{2+}]_i$. Another pathway that regulates insect nACh receptor function involves cGMP through protein kinase G (PKG) activation. In fact, elevation of cGMP stimulates the activity of PKG, which can downregulate insect nACh receptors. In this case, the nitric oxide (NO)-induced stimulation of guanylate cyclase (GC) or variation of the $[Ca^{2+}]_i$ signal can modulate cGMP-dependent PKG activation, which thereby affects nACh receptor responses.

In addition to these complex phosphorylation mechanisms including PKA, PKG or CaMK pathways, two different types of protein kinase C (PKC) (classical and novel PKC, termed PKC1 and PKC2, respectively) can up- and down-regulate insect neuronal nACh receptor functions through changes in $[Ca^{2+}]_i$ and muscarinic receptor activation. Thus, the stimulation of muscarinic receptors by low concentrations of muscarine leads to activation of phospholipase C (PLC) causing hydrolysis of phosphatidylinositol (4,5)-bisphosphate (PtdIns(4,5)$P_2$) into inositol (1,4,5)-trisphosphate (Ins(1,4,5)$P_3$) and diacylglycerol (DAG), which activates PKC1 through an elevation of $[Ca^{2+}]_i$. By contrast, marked elevation of $[Ca^{2+}]_i$ results in a protein phosphatase (PP2B)-induced inhibition of PKC1. In this case, the calcium-independent activation of PKC2 can be used to maintain downregulation of nACh receptor 1 function. These complex regulatory mechanisms of insect nACh receptors, which indicate possible cross-talk between different pathways, have fundamental consequences, particularly for the mode of action of insecticides.

**Table I. Putative phosphorylation sites on insect α7-like subunits between transmembrane domains TM3 and TM4**

| Subunits | Putative phosphorylation sites | |
|---|---|---|
| | PKA and PKG | PKC |
| ***Drosophila melanogaster*** | | |
| Dα5 | 0 | 3 |
| Dα6 | 1 | 4 |
| Dα7 | 0 | 1 |
| ***Heliothis virescens*** | | |
| Hvα7-1 | 0 | 1 |
| Hvα7-2 | 1 | 2 |
| ***Myzus persicae*** | | |
| Mpα7-2 | 0 | 0 |
| ***Apis mellifera*** | | |
| Amα7-1[a] | 0 | 3 |
| Amα7-2[a] | 0 | 1 |
| ***Anopheles gambiae*** | | |
| Agamα5 | 0 | 3 |
| Agamα6 | 1 | 5 |
| Agamα7 | 0 | 1 |
| ***Bemisia tabaci*** | | |
| Btα7-2 | 0 | 2 |

[a]Amα7-1 and Amα7-2 are equivalent to Amelα7 and Amelα5, respectively [61]

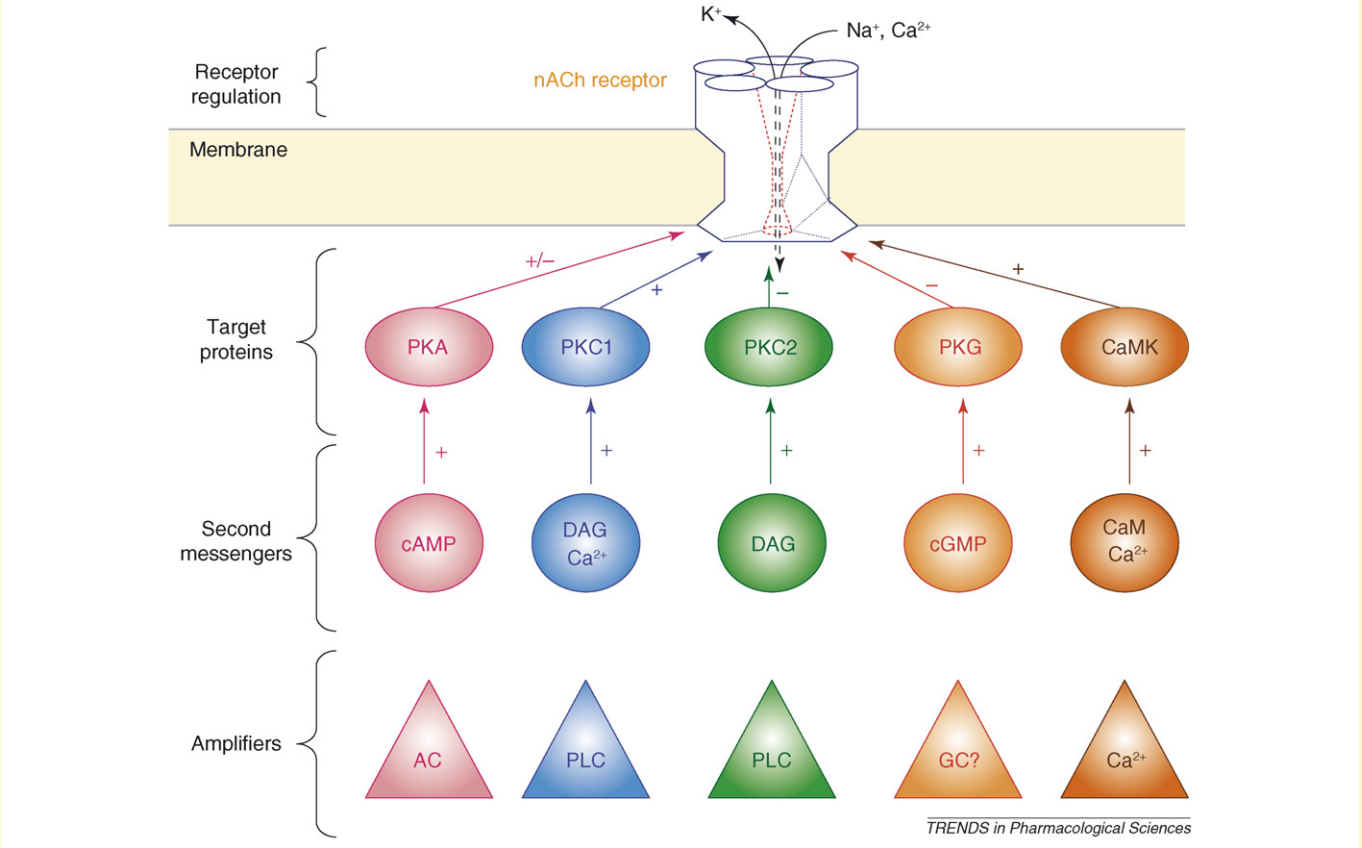


**Figure I.** Upregulation and downregulation of insect nACh receptors. Five different pathways for activating (+) or inhibiting (−) insect nACh receptors are shown. The pathway involving AC and PKA is shown in red. The pathways involving PLC and DAG are shown in blue and green. The pathway represented in blue is sensitive to $[Ca^{2+}]_i$ and involves PKC1. In the pathway shown in green, PKC2 is insensitive to $[Ca^{2+}]_i$ and is involved in the inhibition of nACh receptor 1. A putative pathway that involves cGMP and PKG is shown in orange; this pathway might be activated by GC (indicated by question mark). $[Ca^{2+}]_i$ can act directly through CaMK to upregulate nACh receptor 1 (pathway shown in brown).

imidacloprid and a reduction of the $EC_{50}$ of ACh on the Dα2–β2 hybrid receptor [29]. This indicates that proline has a key role in the selective action of imidacloprid on the Dα2 subunit [30]. Interestingly, although the deletion of the loop B–C interval (Figure 3b) from the Dα2 subunit has little effect on the ACh and the imidacloprid concentration–response curves, the combination of this deletion with the P242E mutation in loop C reduced imidacloprid sensitivity of the Dα2–β2 hybrid receptor [29]. These results provide evidence that insect α subunits possess motifs that modulate the actions of neonicotinoid insecticides, and highlight the role of the loop B–C interval in the neonicotinoid selectivity. Interestingly, this loop varies in its amino acid sequence between insects [29]. The role of loop B in the actions of imidacloprid is exemplified by recent studies on brown planthopper (*Nilaparvata lugens*) [31,32] and cat flea nACh receptors [33]. A field-collected population of *N. lugens*, selected with imidacloprid for 25 generations in the laboratory, developed more than 70-fold greater resistance compared with a susceptible reference strain [31]. Comparison of cDNA sequences of the four α subunits cloned from susceptible and resistant strains revealed that a unique mutation of conserved tyrosine to serine in loop B from Nlα1 and Nlα3 subunits confers resistance to imidacloprid [32]. Although a comprehensive understanding of native receptors carrying these mutations is still lacking, it seems that the loop B–C region in the α subunit [29] and loop D in the non-α subunit have a role in the selectivity of imidacloprid for insect nACh receptors [34].

## Intracellular regulation of insect neuronal nACh receptors

Another novel and interesting feature is the recent characterization of the intracellular regulation of insect nACh receptors. An initial search for patterns of conserved amino acid residues associated with phosphorylation sites in nACh receptor subunits shows that they possess different potential phosphorylation sites for cAMP-dependent protein kinase A (PKA), protein kinase C (PKC), calcium-calmodulin-dependent protein kinase (CaM kinase) and endogenous protein tyrosine kinase [35,36]. Thus, like their vertebrate neuronal nACh receptor counterparts [37,38], insect nACh receptors are regulated by phosphorylation (Box 1). For example, the ACh responses of a cockroach (*Periplaneta americana*) motor neuron are modulated by a cAMP-mediated phosphorylation-dependent intracellular signalling pathway [39]. Moreover, two α-Bgt-sensitive nACh receptor subtypes have been characterized in unidentified thoracic ganglion neurons [40] based on their different rates of desensitization. The 'desensitizing' nACh receptor is selectively inhibited by imidacloprid, and the 'nondesensitized' nACh receptor is selectively blocked by methyllycaconitine [40]. Based on these findings, it has been suggested that the variability of the rate of desensitization of nACh receptor might be caused in part by phosphorylation processes [40]. In the same way, two types of native α-Bgt-insensitive nACh receptors, named nACh receptor 1 and nACh receptor 2, have been characterized in cockroach dorsal unpaired median (DUM) neurons. nACh receptor 1, which is insensitive to imidacloprid, is the only nACh receptor that is modulated by intracellular messengers such as PKA, CaM kinase II and the protein phosphatase PP1–2A [41]. Moreover, its function is upregulated by PKC1 and downregulated by PKC2, which differ in their pharmacological properties and intracellular calcium sensitivity. PKC1, which is activated by the phorbol ester phorbol 12-myristate 13-acetate (PMA), and insensitive to rottlerin, is dependent on intracellular calcium, whereas PKC2, activated by the diacylglycerol analogue DiC8 and inhibited by rottlerin, is calcium-independent [42]. From these results, we suggest that, in contrast to nACh receptor 2, nACh receptor 1 possesses a consensus sequence for intracellular phosphorylation, probably in the transmembrane domain TM3–TM4. Indeed, by comparing all known insect sequences, potential candidates for specific phosphorylation mechanisms emerge. For instance, in *Apis mellifera*, Amα7-1 and Amα7-2 subunits seem to be good candidates for PKC phosphorylation (Box 1). It should be noted that, in the human α7 neuronal nACh receptor, mutation of the conserved tyrosines 386 and 442 to alanine accounts for receptor insensitivity to kinase or phosphatase [37], indicating that functional properties of the receptor depend on the tyrosine phosphorylation status. By contrast, these tyrosines 386 and 442 that are conserved in vertebrate nACh receptor subunits are not conserved in all insect nACh receptor subunits. This might explain in part the different degree of phosphorylation observed between insect nACh receptors. From these studies, it seems that elevated intracellular concentrations of cAMP [41] or cGMP [43] (or both), which stimulate the activity of the respective protein kinases, PKA and PKG, which in turn activate the phosphorylation of consensus sequences, could affect the action of insecticides, as has been previously demonstrated for imidacloprid [41].

## Concluding remarks

Coexpression of insect α subunits with vertebrate β subunits (hybrid nACh receptors), in addition to studies of chimaeric subunits, has provided important new insights into the pharmacological properties of insect nACh receptors [11,28,33,44]. But, in view of the problems associated with heterologous expression, other approaches such as RNA interference and studies of nACh receptor mutants in combination with functional studies will help to resolve the contribution of individual nACh receptor subunit genes to native insect receptors. For example, Dα7 mutants have been identified in the giant fiber of the *Drosophila* central nervous system [7] and could be used to analyse and compare the pharmacological properties of insect α7-like receptors, as has been done in mice [45].

To date, functional recombinant nACh receptors have been obtained only with the *Schistocerca gregaria* Sgα1 subunit, and the resulting currents are of low amplitude [16,17,46]. Reasons for lack of robust expression are complex and can be explained as follow: (i) lack of an appropriate β subunit; (ii) lack of key associated proteins; (iii) failure to find appropriate host cell for expression; or (iv) inappropriate intracellular signalling pathways controlling nACh receptor function. A prime objective for the future will be to identify the key molecular

components and a host cell that will facilitate robust functional expression.

Historically, the problem of insect resistance to insecticides, which arose most commonly from mutation of key amino acids, has been avoided by continually introducing new insect control chemicals. An exciting prospect for insect nACh receptors is to confirm the importance of these residues, particularly in the loop B–C region, to understand imidacloprid selectivity better in other sensitive insect species [47,48]. In addition, we have shown that intracellular phosphorylation of insect nACh receptors affects insecticide sensitivity [41]. Because nACh receptor 1 is phosphorylated by PKA and two different PKCs, we have suggested that nACh receptor 1 possesses potential sites for PKA and PKC between the transmembrane domains TM3 and TM4. Comparative analysis of these potential phosphorylation sites in all insects will help improve understanding of the involvement of phosphorylation in the modulation of insecticide action.

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