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Subchronic exposure to sublethal dose of imidacloprid changes electrophysiological properties and expression pattern of nicotinic acetylcholine receptor subtypes in insect neurosecretory cells

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ABSTRACT

Neonicotinoids are the most important class of insecticides used in agriculture over the last decade. They act as selective agonists of insect nicotinic acetylcholine receptors (nAChRs). The emergence of insect resistance to these insecticides is one of the major problems, which limit the use of neonicotinoids. The aim of our study is to better understand physiological changes appearing after subchronic exposure to sublethal doses of insecticide using complementary approaches that include toxicology, electrophysiology, molecular biology and calcium imaging. We used cockroach neurosecretory cells identified as dorsal unpaired median (DUM) neurons, known to express two α-bungarotoxin-insensitive (α-bgt-insensitive) nAChR subtypes, nAChR1 and nAChR2, which differ in their sensitivity to imidacloprid. Although nAChR1 is sensitive to imidacloprid, nAChR2 is insensitive to this insecticide. In this study, we demonstrate that subchronic exposure to sublethal dose of imidacloprid differentially changes physiological and molecular properties of nAChR1 and nAChR2. Our findings reported that this treatment decreased the sensitivity of nAChR1 to imidacloprid, reduced current density flowing through this nAChR subtype but did not affect its subunit composition (α3, α8 and β1). Subchronic exposure to sublethal dose of imidacloprid also affected nAChR2 functions. However, these effects were different from those reported on nAChR1. We observed changes in nAChR2 conformational state, which could be related to modification of the subunit composition (α1, α2 and β1). Finally, the subchronic exposure affecting both nAChR1 and nAChR2 seemed to be linked to the elevation of the steady-state resting intracellular calcium level. In conclusion, under subchronic exposure to sublethal dose of imidacloprid, cockroaches are capable of triggering adaptive mechanisms by reducing the participation of imidacloprid-sensitive nAChR1 and by optimizing functional properties of nAChR2, which is insensitive to this insecticide.

1. Introduction

Neonicotinoids are the most important class of insecticides used in agriculture over the last decade and are effective against some crop pests such as aphids, thrips and whiteflies. Imidacloprid was the first product of this class of insecticides to be commercialized in 1991 and it was used in foliar application and seed treatments (Tomizawa and Casida, 2003). Neonicotinoids act as selective agonists of insect nicotinic acetylcholine receptors (nAChRs) (Tomizawa and Casida, 2005), which belong to the "cys-loop" superfamily of ligand-gated ion channels (Ffrench-Constant et al., 2016). These receptors are composed of five subunits (Jones et al., 2007), each subunit possesses four transmembrane domains (M1-M4), an extracellular amino-terminal domain involved in agonist binding and a large cytoplasmic loop between M3 and M4 containing several phosphorylation sites (Dupuis et al., 2012). Subunits were classified into two groups α and non α or β, depending on the presence or not of two adjacent cysteine residues in the extracellular domain, which play an important role for acetylcholine binding (Jones et al., 2007). In insects, several nAChR subunits have been cloned and the sequencing of the entire insect genome has revealed the existence of approximately ten different

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nAChR subunit genes (Jones and Sattelle, 2010) suggesting a huge number of hypothetical nAChR subtypes. Combinations of nAChR subunits result in distinct receptors, with their own electrophysiological and pharmacological properties, which thereby influence sensitivity to neonicotinoids (Lansdell and Millar, 2000; Millar and Lansdell, 2010). In addition, previous studies have shown that neonicotinoid efficacy on nAChR subtypes depends on electropharmacological properties and many cellular and molecular factors such as conformational state, membrane potential, subunit composition and calcium-dependent phosphorylation/dephosphorylation process (Courjaret and Lapied, 2001; Bodereau-Dubois et al., 2012; Calas-List et al., 2013; List et al., 2014; Salgado, 2016; Sun et al., 2016).

However, despite this specific activity, one major problem, which may threaten the use of neonicotinoids is the emergence of insect resistance to these insecticides (Bass et al., 2015; Ffrench-constant et al., 2016). In the case of neonicotinoids, resistance observed in several insect species was initially attributed to metabolic mechanisms through modifications of the detoxification enzyme expressions. Latter, target-site resistance to neonicotinoids was also described (Liu et al., 2005; Slater et al., 2012; Casida and Durkin, 2013; Bass et al., 2015) and finally, very recent studies have suggested that quantitative changes in nAChR subunits may also contribute to target-site resistance to neonicotinoids (Zhang et al., 2015).

In the general context of the effectiveness of pest insect resistance management, our aim is to use cockcroaches as model to better understand physiological changes appearing after subchronic exposure to sublethal dose of a neonicotinoid, imidacloprid. Although previous studies have explored the effects of sublethal doses of neonicotinoids in insects, they were mainly focused on the behavioral effects (e.g., locomotor activity and impairs olfactory learning and memory), especially in non-target insects, such as honey bees (Aliouane et al., 2009; Blacquière et al., 2012; Tan et al., 2015; Mengoni Goñalons and Farina, 2015). Up to date, there are no data related to the effects of subchronic exposure to sublethal dose of neonicotinoids on both physiological and molecular features of insect nAChRs. For that purpose, cockroach *Periplaneta americana* neurosecretory cells identified as dorsal unpaired median (DUM) neurons, known to express two distinct α-bgt-insensitive nAChR subtypes named nAChR1 and nAChR2 (Courjaret and Lapied, 2001; Bodereau-Dubois et al., 2012), have been used. Previous findings have reported that nAChR subtypes present different pharmacological properties. Although nAChR1 is sensitive to the neonicotinoid imidacloprid, nAChR2 is insensitive to this insecticide, whereas the insect has never been exposed to this insecticide (Courjaret and Lapied, 2001). Furthermore, we have demonstrated that the uncommon conformational state of nAChR2 (i.e., open at the resting state and closed upon cholinergic agonist application) (Courjaret and Lapied, 2001; Courjaret et al., 2003; Bodereau-Dubois et al., 2012) is responsible for the different neonicotinoid sensitivity observed in these two nAChR subtypes. Consequently, because cockroach neuronal preparations together with DUM neurons are commonly used as biological models for vertebrates and invertebrates to study the mode of action of neurotoxic insecticides (Pelhate et al., 1990), these interesting features make DUM neuron nAChR1 and nAChR2 subtypes a suitable model to explore the influence of subchronic exposure to sublethal dose of imidacloprid on both physiological and molecular properties of insect nAChRs. Our study reports that the subchronic exposure of cockroaches *Periplaneta americana* to sublethal dose of imidacloprid, differently affect electropharmacological properties and subunit expression pattern of DUM neuron nAChR1 and nAChR2 subtypes, which thereby impact their physiological functions. These results provide additional information that may contribute to better understand the mechanisms underlying the development of insect resistance to insecticides.

2. Materials and methods

All experiments were performed on adult male cockroaches *Periplaneta americana* taken after the last-instar nymph stage from our laboratory stock colony, which are maintained under standard conditions (29 °C, photo-cycle 12 h light/12 h dark).

2.1. Exposure to imidacloprid

Imidacloprid (Sigma-Aldrich, Saint Quentin Fallavier, France) was resuspended in dimethyl sulfoxide (DMSO) to obtain a stock solution at 100 mg ml^{-1}. Subsequent dilutions of imidacloprid were prepared in sucrose syrup (10% sucrose solution w/v) for the cockroach exposure experiments. Cockroaches were deprived of access to water for 48 h. Insects were then exposed to imidacloprid by ingesting 10 μl of sucrose syrup containing the different doses of imidacloprid ranging from 0.01 μg to 30 μg/cockroach. Control experiments were performed under the same experimental conditions without imidacloprid. Mortality rate was assessed 48 h after the treatment. We used 30–40 cockroaches per dose. For subchronic exposure to sublethal dose experiments, 30 cockroaches were daily and orally exposed *ad libitum* 30 days to the highest dose of imidacloprid that did not produce significant mortality. Control groups were similarly treated without imidacloprid.

2.2. Electrophysiological recordings

2.2.1. Cell preparation

Patch-clamp recordings were performed on DUM neuron cell bodies isolated from the midline of the terminal abdominal ganglion (TAG) of the nerve cord of the treated and non-treated adult male cockroaches. The TAG were removed from the nerve cord and placed in cockroach saline containing 200 mM NaCl, 3.1 mM KCl, 5 mM $CaCl_2$, 4 mM $MgCl_2$, 10 mM HEPES and 50 mM sucrose, pH was adjusted to 7.4 with NaOH. Isolation of DUM neuron cell bodies was performed under sterile conditions after enzymatic digestion and mechanical dissociation, as previously described (Lapied et al., 1989). DUM neuron cell bodies were maintained at 29 °C for 24 h before electrophysiological experiments were carried out.

2.2.2. Whole-cell recording

Nicotine- and imidacloprid-induced currents were recorded by using the patch-clamp technique in the whole-cell recording configuration under voltage-clamp mode, at a steady-state holding potential of −50 mV except when otherwise stated. Input membrane resistances were recorded under current-clamp condition in response to a hyperpolarizing current pulse (150 pA in amplitude and 300 ms in duration). Signals were recorded with an Axopatch 200A patch-clamp amplifier (Axon instruments), digitized and acquired using a MiniDigidata 1440 analog-digital converter (Axon Instruments). Currents were treated with axoscope 10.2 software (Axon Instruments). Patch pipettes were pulled from borosilicate glass capillary tubes (GC 150T-10; Clark Electromedical Instruments, Harvard Appartus Edenbridge, UK) using a P-97 Flaming/Brown Micropipette Puller (Sutter Instrument Company, Novato, U.S.A). Pipettes had resistances ranging from 1 to 1.5 MΩ when filled with internal pipette solution (see composition below). The liquid junction potential between bath and internal solutions was always corrected before the formation of a gigaohm seal (>1 GΩ). Ionic currents induced by nicotine and imidacloprid were recorded with software control pClamp