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Novel nicotinic action of the sulfoximine insecticide sulfoxaflor

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ABSTRACT

The novel sulfoximine insecticide sulfoxaflor is as potent or more effective than the neonicotinoids for toxicity to green peach aphids (GPA, *Myzus persicae*). The action of sulfoxaflor was characterized at insect nicotinic acetylcholine receptors (nAChRs) using electrophysiological and radioligand binding techniques. When tested for agonist properties on *Drosophila melanogaster* Dα2 nAChR subunit co-expressed in *Xenopus laevis* oocytes with the chicken β2 subunit, sulfoxaflor elicited very high amplitude (efficacy) currents. Sulfoximine analogs of sulfoxaflor were also agonists on Dα2/β2 nAChRs, but none produced maximal currents equivalent to sulfoxaflor nor were any as toxic to GPAs. Additionally, except for clothianidin, none of the neonicotinoids produced maximal currents as large as those produced by sulfoxaflor. These data suggest that the potent insecticidal activity of sulfoxaflor may be due to its very high efficacy at nAChRs. In contrast, sulfoxaflor displaced [^{3}H]imidacloprid (IMI) from GPA nAChR membrane preparations with weak affinity compared to most of the neonicotinoids examined. The nature of the interaction of sulfoxaflor with nAChRs apparently differs from that of IMI and other neonicotinoids, and when coupled with other known characteristics (novel chemical structure, lack of cross-resistance, and metabolic stability), indicate that sulfoxaflor represents a significant new insecticide option for the control of sap-feeding insects.

1. Introduction

New insecticides are needed for the control of sap-feeding insects due, in part, to the development of resistance to current insecticides resulting from their broad-scale use. Insecticides acting at insect nicotinic acetylcholine (ACh) receptors (nAChRs) have proven particularly useful for the control of aphids and whiteflies, especially the neonicotinoid class (Fig. 1), which represented nearly one-fourth of all insecticide sales globally in 2007 (Jeschke and Nauen, 2008; Babcock et al., 2011). Imidacloprid (IMI, Fig. 1) is the most used neonicotinoid insecticide, and therefore the standard for comparison.

Neonicotinoid insecticides exert their insecticidal effects through a selective interaction with insect nAChRs (Jeschke and Nauen, 2008). The functional properties of the neonicotinoid insecticides have been studied using both native and cloned nAChR subunits. To date, the commercialized neonicotinoid insecticides have been shown to be agonists, or partial agonists of insect nAChRs (Bai et al., 1991; Matsuda et al., 1998; Kagabu et al., 2002; Nishiwaki et al., 2003; Tan et al., 2007). Additionally, clothianidin (Fig. 1) produces very large amplitude currents, and was described as a "super-agonist" of insect nAChRs (Ihara et al., 2003a, 2003b, and Brown et al., 2006). While the precise role of larger amplitude currents relative to insecticidal activity has not yet been determined, a relationship between maximal neonicotinoid currents (efficacy) and cockroach toxicity has been proposed (Tan et al., 2007). [^{3}H]IMI has become an established tool in mode of action research for radioligand displacement studies of insect nAChRs (Liu and Casida, 1993).

A novel chemotype containing a sulfoximine moiety has outstanding insecticidal activity (Zhu et al., 2005). Structure optimization led to the discovery of the insecticide sulfoxaflor (Fig. 2) with potent, broad spectrum activity for sap-feeding pests (Babcock et al., 2011; Zhu et al., in press). Sulfoxaflor has thus far proven to lack cross-resistance in strains of whiteflies and brown planthopper that are likely resistant to the commercial neonicotinoid insecticides due to metabolic detoxification mechanisms (Babcock et al., 2011; Zhu et al., in press). The sulfoximine functionality may impart unique chemical properties, including stability to metabolism in neonicotinoid resistant insects. Alternatively, the differences in chemical structure between sulfoxaflor and the neonicotinoids could result in a differential interaction with nAChRs.

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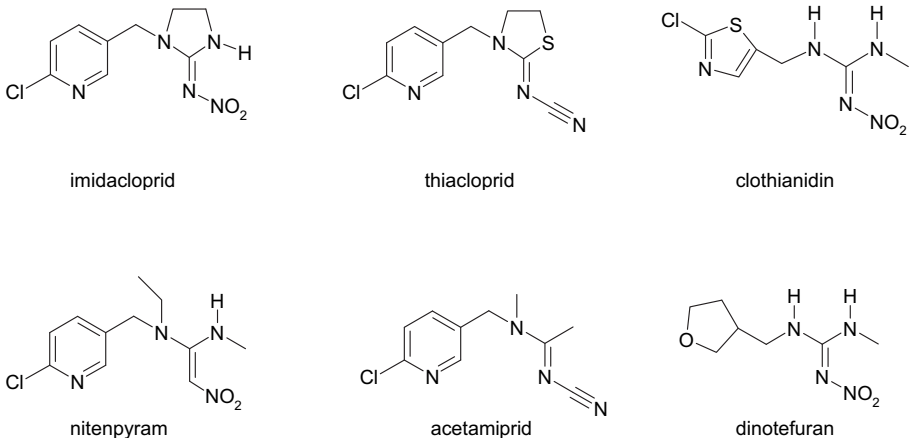

Fig. 1. Neonicotinoids used in this study.

The present study investigates the activity of sulfoxaflor and related sulfoximines at nAChRs using established electrophysiological techniques (*Drosophila melanogaster* α nAChR subunits co-expressed with chicken β2 subunits) and [^{3}H]IMI displacement from green peach aphid (GPA, *Myzus persicae*) membranes. The activity of several commercial neonicotinoid insecticides is compared with that of sulfoxaflor in these assays. Our study indicates that sulfoxaflor elicits very high efficacy (amplitude) currents from nAChRs expressed in oocytes, but shows relatively weak affinity for the IMI nAChR binding site. This combination of electrophysiological and binding characteristics is distinct from the commercial neonicotinoids, and to some extent from that of other insecticidal sulfoximines lacking the entirety of the structure of sulfoxaflor. We propose that the novel sulfoxaflor-nAChR interaction is uniquely different than that of the neonicotinoids.

2. Materials and methods

2.1. Chemicals

Sulfoxaflor and related sulfoximines were synthesized at Dow AgroSciences. IMI, thiacloprid, clothianidin, dinotefuran, and acetamiprid were purchased from Chem Service, Inc. (West Chester, PA), nitenpyram from Waterstone Technology (Carmel, IN), and all other chemicals from Sigma Chemical (St. Louis, MO). [^{3}H]IMI was prepared by Amersham (Piscataway, NJ) and had a specific activity of 37 Ci/mmol.

2.2. Toxicity tests

The toxicity of test compounds to GPAs was assessed as previously described (Babcock et al., 2011). Briefly, seedling cabbage plants (*Brassica oleracea capitata*) were infested with equivalent numbers of GPA. One day following infestation, plants were sprayed on all leaf surfaces with test compounds dissolved in methanol: 20% acetone (1:1). Tween 20 (0.27 mg/L) was added to all test solutions. Treated plants were held at 25 °C (16:8 h light:-dark) and 3 days after treatment live aphids on each plant were counted. Data were corrected for control mortality using Abbott's formula (Abbott, 1925). Dose-response relationships were analyzed using log dose probit regression (Finney, 1971). In some studies, leaves were cut from infested and treated plants and the symptoms of GPA intoxication were assessed under a stereomicroscope.

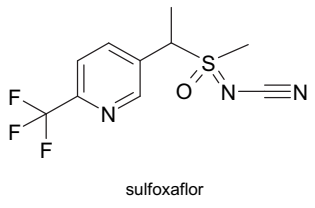

Fig. 2. Structure of sulfoxaflor.

2.3. RT-PCR of Dα1, Dα2, and chicken β2

The *D. melanogaster* α1 nAChR subunit (Dα1) was amplified from 1st strand cDNA synthesized from larval mRNA (Clontech Laboratories, Mountain View, CA) using SuperScript™ III reverse transcription kit (Invitrogen, Carlsbad, CA). PCR was performed with the primers DALSfor (5′-AAGCTTACCATGGGTAGCGTGCTATTC-3′) and DALSrev (5′-AAGCTTCTATAAGGTGTTCTCGCTG-3′) and AccuPrime™ polymerase (Invitrogen, Carlsbad, CA). Cycling conditions were: 94 °C/30 s, 51.2 °C/30 s, 68 °C/2 min for 30 cycles. A 1719 bp product was purified with the Qiaex II Gel Extraction Kit (Qiagen, Inc., Valencia, CA). The purified 1719 bp PCR product was ligated into pCR2.1-TOPO and several clones containing the Dα1 gene were sequenced. A clone identical to the published sequence (GenBank accession number NM_079757) was excised from the TOPO vector with Hind III and ligated into pGH19 which was linearized with Hind III. A clone with Dα1 inserted in the correct orientation was identified by restriction analysis.

The *D. melanogaster* α2 nAChR subunit (Dα2) was amplified from 1st strand cDNA synthesized from embryonic mRNA (Clontech) using the primers SADFW2 (5′ AGATCTCACCATGGCTCCTGG CTGCTGCAC 3′) and SADRV2 (5′ AGATCTTTAATTCTTCTTCTCGGTTA 3′). PCR was performed using the FailSafe™ PCR kit (Epicentre Biotechnologies, Madison, WI). A 1747 bp product was amplified, gel purified, and ligated into pCR-BluntII-TOPO. Several clones containing the Dα2 gene were sequenced. One clone having a sequence similar to GenBank accession number X53583 was identified. The clone had two conservative single base changes relative to the published sequence. This clone was isolated as a Bgl II fragment and ligated into pGH19. A clone having the Dα2 in the correct orientation was identified by restriction analysis.

The chicken β2 nAChR subunit (β2) was amplified from 1st strand cDNA made from chicken brain mRNA obtained from Clontech. PCR was performed using TaKaRa EX Taq™ (TaKaRa Bio, Inc, Otsu, Japan) in combination with LA buffer in a reaction using the following cycling conditions; 98 °C/10 s, 55 °C/30 s, 72 °C/1.5 min