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Rapid communication

Mutations in a putative octopamine receptor gene in amitraz-resistant cattle ticks

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Abstract

The mode of action of amitraz is thought to be its toxic effects on a receptor for a neuromodulator, octopamine. Resistance could arise from modifications of this receptor so that it would not be affected by amitraz. A putative octopamine receptor cDNA was cloned and sequenced from a cattle tick in Australia. However, when the sequence was compared between Australian strains of amitraz-susceptible and resistant ticks, no differences were detected. We have sequenced this putative octopamine receptor gene in tick strains from America. The American ticks have a sequence almost identical to that of the Australian ticks with no deletions or additions in the open reading frame. In a Brazilian strain and a Mexican strain that are very resistant to amitraz, there are two nucleotide substitutions that result in amino acids different from all the susceptible strains. Discovery of these mutations only in amitraz-resistant ticks provides the first evidence for the possibility of an altered pesticide target site as a mechanism of amitraz resistance in ticks.
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1. Introduction

The southern cattle tick, *Rhipicephalus (Boophilus) microplus* is a bloodsucking ectoparasite that is one of the most important pests of cattle in tropical and sub-tropical regions of the world. It causes significant losses to the livestock industry from its direct effects on production and more importantly, from its transmission of deadly pathogens such as *Babesia* and *Anaplasma* (Young et al., 1988). Although *R. microplus* was eradicated from the southern United States, it is still a major problem in Mexico, and the re-infestation of the U.S. is highly possible. The southern cattle tick has been kept in check primarily with chemical acaricides. Amitraz is one of the acaricides that has been efficacious against *R. microplus* but there is evidence of this tick developing resistance to amitraz (Reid, 1989; Soberanes et al., 2002; Li et al., 2004). Knowledge of the resistance mechanism(s) would lead to the development of more rapid and accurate diagnostic tools for detecting resistance and steer development of alternative acaricides. The current standard FAO larval packet assay (Stone and Haydock, 1962) for assessing acaricide resistance is both slow and labor intensive. Furthermore, an understanding of resistance mechanisms might help circumvent the resistance problem.

The mode of action of formamidine pesticides such as amitraz in insects is believed to be the toxic effects on a G protein-coupled receptor for a neuromodulator,

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octopamine (Evans and Gee, 1980; Dudai et al., 1987). Little is known about the mode of action of or mechanism of resistance to formamadines in ticks and mites. One common pesticide resistance mechanism of arthropods is mutations in the pesticide target rendering the target site insensitive to the pesticide (Hemingway and Ranson, 2000; ffrench-Constant et al., 2000; Tan et al., 2005). A putative octopamine receptor cDNA was isolated from the Australian *R. microplus* (Baxter and Barker, 1999). However, the nucleotide sequence of this cDNA from various Australian amitraz-resistant and amitraz-susceptible cattle ticks was identical. Thus, resistance to amitraz in Australian strains of the cattle tick does not appear to be a result of a point mutation in this putative octopamine receptor gene.

Unlike the Australian ticks, some amitraz-resistant strains of *R. microplus* isolated from the western hemisphere do exhibit mutations resulting in alteration of the amino acid sequence of the putative target site for amitraz. Discovery of these mutations provides the first evidence of target site modification in amitraz-resistant *R. microplus* and enables further studies of the resistance mechanism in these important ticks.

2. Materials and methods

2.1. Ticks

The southern cattle tick was collected from various regions in North, Central and South America. All are maintained at the Cattle Fever Tick Research Laboratory (CFTRL), Edinburg, Texas. Gonzalez (G) is a strain originally collected in 1984 at an outbreak in Zapata County, Texas. It is susceptible to all acaricides tested and was routinely used as a reference strain. The Santa Luiza (SL) strain was originally collected in an area of Brazil where amitraz resistance was suspected. A colony was established at the Centro Nacional de Servicios de Constatacion en Salud Animal, Jiutepec, Morelos, Mexico, and subsequently shipped in November 2000 to CFTRL, where it is maintained and further selected for amitraz resistance. The SL strain has a resistance index of approximately 50 relative to the G strain (Li et al., 2004). The San Alfonso strain was collected in Mexico and is very resistant to amitraz (Soberanes et al., 2002). The Pesqueria strain has low level of amitraz resistance, with resistance index of four relative to the G strain (Li et al., 2004). Coatzacoalcos, Tuxpan and Corrales strains were susceptible to amitraz and their origin has been described previously (He et al., 1999a).

2.2. Amplification and sequencing of the putative octopamine receptor cDNA

Total RNA was extracted from 2-week old unfed larvae with RNeasy® Midi kit (Qiagen, Valencia, CA). First-strand cDNA was synthesized using ThermoScript™ RT-PCR system (Invitrogen, Carlsbad, CA) and a primer designed according to the noncoding 3′ region of the Australian putative octopamine receptor gene (OAR-R1883, 5′-AGA CGT GCG ACG CAC AAG-3′). Oligonucleotides were designed from both noncoding 5′, 3′ regions and open reading frame and used as primers in PCR to amplify the first strand cDNA such that the amplified fragments would overlap (OAR-F171, 5′-GGT TCA CCC AAC CTC ATC TCT GAA-3′; OAR-R1195, 5′-GAC GAC GGG TGA CGT TCT TG-3′; OAR-F1065, 5′-ATC TTT CTG GCA ACC CGA CG-3′; OAR-R1684, 5′-GCA TCA CAG CGA CAG CTC TTC-3′). Amplification was performed in 50 μl of reaction mixture containing, in addition to cDNA, Advantage™ cDNA polymerase, 1× cDNA polymerase buffer, 0.2 mM each of dNTPs (all from Clontech, Palo Alto, CA), and 0.2 μM of each primer. The cycling conditions for amplifying both cDNA fragments are 94 °C for 1 min, 40 cycles of 94 °C, 30 s, 68 °C, 75 s, and 68 °C for an additional 5 min. The PCR products were purified with NucleoTrap (Clontech) and sequenced using the BigDye™ dye terminator and the PRISM® 3100 genetic analyzer (both from Applied Biosystems, Foster City, CA). All thermal cycling was carried out in a PTC-200 DNA Engine (MJ Research, Watertown, MA).

2.3. Genomic DNA sequencing

DNA was extracted from individual ticks using 100 μl 5% Chelex (Bio-Rad, Hercules, CA) in 0.1× TE buffer (pH 8.0) following manufacturer's instruction. A 417 bp fragment of DNA containing the mutations was amplified using OAR-F171 and OAR-R587 (5′-GCA GAT GAC CAG CAC GTT ACC G-3′). PCR was performed as described above in 20 μl reactions at 94 °C for 1 min, 40 cycles of 94 °C, 5 s, 68 °C, 60 s, and 68 °C for an additional 5 min. After size verification on agarose gels, PCR products were treated with ExoSAP-IT (USB Corp., Cleveland, OH) according to manufacturer's instructions and sequencing was performed as described above.

3. Results and discussion

We compared the cDNAs sequenced from American amitraz-susceptible strain (G) and amitraz-resistant