**Insecticidal activity of oxadiazine insecticide indoxacarb and its \( N \)-decarbomethoxylated metabolite and their modulations of voltage-gated sodium channels**

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Abstract
Insecticidal bioassays on *Plutella xylostella* (Linneaus) of an oxadiazine insecticide indoxacarb and its \( N \)-decarbomethoxylated metabolite (DCJW), and their modulation of voltage-gated sodium channels in rat dorsal root ganglion neurons were examined. No significant difference was observed in insecticidal activity between indoxacarb and DCJW in ingestion and contact tests. In patch-clamp experiments, both indoxacarb and DCJW suppressed the peak sodium currents in a time- and dose-dependent manner. DCJW at 1 \( \mu \)M blocked the sodium currents to 62.7±3.0% (\( n=5 \)) of the control after 25 min of bath application. In contrast, in the presence of 1 \( \mu \)M indoxacarb, currents were blocked to 4.5±0.6% (\( n=4 \)) of control. Thus, the potency of DCJW in blocking sodium currents was higher than that of indoxacarb in rat dorsal root ganglion neurons.

Key words: Indoxacarb, insecticidal activity, *Plutella xylostella*, voltage-gated sodium channel, whole-cell patch clamp

**INTRODUCTION**

An oxadiazine insecticide indoxacarb has been developed for controlling a broad spectrum of pest insects on various crops (Harder et al., 1996). Patch-clamp experiments clearly demonstrated that indoxacarb suppressed the voltage-gated sodium currents (Nagata et al., 1998). Wing et al. (1998) reported that indoxacarb is metabolized to decarbomethoxylated indoxacarb (DCJW) in several species of lepidopteran larvae and DCJW blocked action potentials in motor neurons in *Manduca sexta*. There is no report for the effects of DCJW at ion channel level.

Rat dorsal root ganglion neurons are endowed with tetrodotoxin-sensitive (TTX-S) and tetrodotoxin-resistant (TTX-R) sodium channels. The TTX-R sodium channels exhibit slower ion channel kinetics compared to those of the TTX-S sodium channels (Roy and Narahashi, 1992). The mechanism of fast interaction of insecticides with ion channels may take place in the order of milliseconds. Thus, the TTX-R sodium channel is a relevant tool to study the effects of insecticides on sodium channels in detail (Tatebayashi and Narahashi, 1994; Song and Narahashi, 1996).

In the present study, the effects of indoxacarb and DCJW on the TTX-R voltage-gated sodium channels were examined using a whole-cell patch-clamp technique in rat dorsal root ganglion neurons. The relative potency of indoxacarb and DCJW on the TTX-R sodium channels was compared with the insecticidal activity of these chemicals on *Plutella xylostella*. Results indicate that DCJW was more potent than indoxacarb in blocking sodium currents in rat dorsal root ganglion neurons. However, there was no significant difference in the insecticidal activity between the two chemicals.

**MATERIALS AND METHODS**

Chemicals. Indoxacarb (>99% purity) and DCJW (>99%) were provided by Du Pont Agri-
cultural Products (Newark, DE, U.S.A., Fig. 1). These chemicals have one asymmetric carbon and two optical isomers exist. Both compounds used in the present study were 50:50 mixtures of (R)- and (S)-isomers. The compounds were dissolved as stock solution in acetone for bioassay and DMSO for patch clamp experiments.

Bioassay. For ingestion test, young cabbages at the 5- to 7-leaf stage were sprayed with different concentrations of compounds in acetone–water (75:25) mixed solvent and allowed to dry in a draft chamber. Controls were treated with solvent only. Cabbage leaves were placed into a plastic cup with a lid (100 mm in diameter, 40 mm in depth), then infested with *P. xylostella*. For contact test, test chemicals diluted in 0.5 ml of acetone were topically applied on the dorsal thorax of *P. xylostella* using a repeating solution dispenser (Hamilton Co., Reno, NE, U.S.A.). Control insects were treated with acetone only. Insects were placed into the plastic cup that contained an untreated cabbage leaf.

The insects were kept at 27°C, and scored for death 48 h after treatment. Insects that did not respond to pencil tip prodding were considered as dead. Ten insects replicated three times were used at each dose level. All data were pooled to estimate the parameters. Data were analyzed by the Probit method (Finney, 1971) using a computer program (LDWIN 0.94a).

Cell preparation. Dorsal root ganglion neurons isolated from newborn rats (4–8 days postnatal) were used by a procedure described previously (Roy and Narahashi, 1992; Nagata and Narahashi, 1994). In short, the spinal columns were removed from anesthetized rats and cut longitudinally. Dorsal root ganglia were plucked from between the vertebrae of the spinal column and were placed immediately in Ca2+ and Mg2+-free phosphate-buffered saline (PBS, GIBCO BRL, Grand Island, NY) solution supplemented with 6 g/l glucose. The ganglia were enzymatically digested in PBS solution containing trypsin (2.5 mg/ml, type XI, Sigma, St. Louis, MO) for 15 min at 37°C. Following the enzyme treatment, ganglia were rinsed with Dulbecco’s Modified Eagle’s Medium (DMEM, GIBCO BRL) supplemented with newborn calf serum (10%, v/v, GIBCO BRL) and gentamicin (80 μg/ml, GIBCO BRL). Cells were mechanically dissociated by repeated triturations using a fire-polished Pasteur pipette and plated on poly-L-lysine coated coverslips (Becton Dickinson Labware, Bedford, MA). Cells were incubated in DMEM supplemented with newborn calf serum (10%, v/v) and gentamicin (80 μg/ml) for 1 to 8 h for patch-clamp experiments (90% air–10% CO2, 37°C).

**Electrophysiological recording.** Sodium currents were recorded by the whole-cell patch-clamp technique. Pipette electrodes (1.0–1.5 MΩ) were made of borosilicate glass capillary (0.8–1.0 mm inner diameter, Kimble Products, Vineland, NJ) using a multi-step horizontal puller (P-97, Sutter Instrument Co., Novato, CA). The internal pipette solution contained (in mM): CsF 135, NaCl 10, and HEPES-acid 5. The pH was adjusted to 7.0 with CsOH, and the osmolarity was 275 mOsm. The external solution contained (in mM): NaCl 25, tetramethylammonium (TMA) chloride 75, tetraethylammonium (TEA) chloride 20, CsCl 5, CaCl2 1.8, MgCl2 1.0, and HEPES-acid 5. The solution was adjusted to pH 7.4 with TEA-OH and 290 mOsm with sucrose. Tetrodotoxin (0.2 μM) was added to the external solution to block the TTX-S sodium currents. An Ag-AgCl pellet/3 M KCl-agar bridge was used for the reference electrode. Membrane currents passing through the pipette were recorded with an Axopatch 200B amplifier (Axon Instruments, Burlingame, CA). Signals were filtered with a low-pass Bessel filter with a cut-off frequency at 5 kHz and stored onto a computer hard disk using Pulse+Pulse Fit 8.11 (HEKA, Lambrecht, Germany). Seventy to 80% of series resistance was compensated. Capacitive and leakage currents were digitally subtracted by using the P+P/4 procedure (Bezanilla and Armstrong, 1977). The liquid junction potential between the internal and external solution was −5.3 mV on average in the present study. All data shown in this paper were compen-
sated for the liquid junction potential.

For application of indoxacarb and DCJW, glass tubing was used in the present study. Details for the perfusion method have been described (Tatebayashi and Narahashi, 1994). The DMSO concentration in the perfusate was <0.1%. DMSO at this concentration had no effect on the sodium currents when applied externally.

RESULTS

Insecticidal activity of indoxacarb and DCJW

LC$_{50}$ values of indoxacarb and DCJW in the ingestion study were estimated to be 0.69 and 0.74 $\mu$m, respectively. In the contact study, LD$_{50}$ values of indoxacarb and DCJW were estimated to be 84.2 and 93.8 pmol/insect, respectively (Table 1). Thus, there was no significant difference in insecticidal activity between indoxacarb and DCJW either by ingestion or by contact.

Effects of indoxacarb and DCJW on sodium channel currents

In rat dorsal root ganglion neurons, two types of cells could be distinguished in terms of their sensitivity to TTX (Roy and Narahashi, 1992). In the present study, all cells used in the patch clamp experiments were resistant to TTX and the sodium currents evoked by depolarizing pulses were not blocked by 0.2 $\mu$m TTX. When currents were evoked by a step depolarization to 0 mV from a holding potential of $-90$ mV, a typical inward TTX-R sodium current was observed. The currents were maintained at a stable level over a period of up to 1 h in the control. When indoxacarb or DCJW at 10 $\mu$m were perfused into the application chamber, these chemicals exerted suppressive effects on the TTX-R sodium currents in a time-dependent manner (Fig. 2). The peak currents were suppressed slowly and continuously until complete suppression was achieved (data not shown).

Figure 3 shows the effects of indoxacarb and DCJW on the TTX-R sodium currents in two different perfusing concentrations of the compounds. The percentage of peak current relative to control was drawn in the histogram. The suppressive effects were determined by measuring the peak amplitude at 25 min after application. Indoxacarb and DCJW at 1 $\mu$m blocked 4.4±0.6% ($n=4$) and 62.7±3.0% ($n=5$) of the control sodium currents, respectively. At a concentration of 10 $\mu$m, indoxacarb and DCJW blocked 46.6±3.0% ($n=4$) and 76.9±3.4% ($n=5$) of the sodium currents, respectively. Thus, DCJW was more potent in blocking the sodium currents than indoxacarb in rat dorsal root ganglion neurons.

Table 1. Insecticidal activity of indoxacarb and DCJW on the diamondback moth, *Plutella xylostella* (Linnaeus)*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ingestion</th>
<th>Contact</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>LC$_{50}$ ($\mu$m)</td>
<td>LD$_{50}$ (pmol/insect)</td>
</tr>
<tr>
<td>Indoxacarb</td>
<td>0.69 (0.46–1.04)</td>
<td>84.2 (57.1–126.3)</td>
</tr>
<tr>
<td>DCJW</td>
<td>0.74 (0.48–1.15)</td>
<td>93.8 (60.4–154.3)</td>
</tr>
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</table>

*Experiments were replicated three times. Data were pooled for Probit analysis, and then median lethal concentration (LC$_{50}$) and dose (LD$_{50}$) were estimated. 95% confidence range is shown in parentheses.
DISCUSSION

Previous reports have demonstrated that orally administered indoxacarb and DCJW showed identical levels of insecticidal activity in larval *M. sexta* (Wing et al., 1998). Indoxacarb showed no contact effects on adult *Spodoptera littoralis* (Pluschkell et al., 1998). Wing et al. (1998) proposed the idea that orally administered indoxacarb was bioactivated to DCJW, which could explain the no contact effect of indoxacarb. In the present study, both indoxacarb and DCJW showed contact and oral activities on *P. xylostella* larvae and there was no significant difference in the insecticidal activity between the two chemicals.

It is possible that a difference in hydrophobicity could account for differences in toxicity and neuronal effects between the two chemicals. However, for indoxacarb and DCJW, there was no significant difference in hydrophobicity when estimated as octanol-water partition coefficient (Wing et al., unpublished data). We have found that there was no difference in cuticular penetration between the two chemicals in adult *Musca domestica* (Sugiyama et al., 2001).

One possible reason could be that the metabolic pathways which affect the bioactivation in *P. xylostella* may be different from that of *S. littoralis*. The amount of DCJW in the body was more than that of indoxacarb in *M. domestica* (Sugiyama et al., 2001). Further studies on the detailed metabolic pathway of indoxacarb are warranted to clarify the activation mechanisms of indoxacarb.

Indoxacarb and DCJW showed clear differences in suppressive effects on voltage-gated sodium currents in rat dorsal root ganglion neurons. The hydrophobicity of neuroactive substances might affect the time course of their effects on ion channels. However, as described above, there was no difference in hydrophobicity between indoxacarb and DCJW. Thus, the differences observed in the present study at ion channel level could have been caused by a difference in efficacy at the site of action.

One possible explanation for the inconsistent potency between the insecticidal activities in *P. xylostella* and the effects on sodium channels in rat dorsal root ganglion neurons is that there could be differences in the sensitivity of sodium channels to chemicals between insects and mammals. Patch clamp experiments using insect neurons are warranted to verify this hypothesis.

Another possible reason for the inconsistency is that the voltage-gated sodium channel is not a primary target site of action. We have shown previously that indoxacarb potently modified the kinetics of neuronal nicotinic acetylcholine receptors in rat cortical neurons at physiological concentration (Zhao et al., 1999). Indoxacarb accelerated the desensitization of acetylcholine-induced currents. In contrast, DCJW had no effects on nicotinic acetylcholine receptors. This data indicates that indoxacarb has multiple target sites in neuronal ion channels with different potencies. More detailed studies at ion channel level are warranted to clarify the mode of action of indoxacarb and DCJW.

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REFERENCES


Finney, D. J. (1971) *Probit Analysis*. 3rd ed. Cambridge Uni-


