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Azadirachtin and Cypermethrin Induced Alterations in Electrophysiological Properties of Sensory and Interneurons in *Periplaneta americana*

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Synthetic pyrethroid compounds are neurotoxic insecticides (Coats 1990). These pesticides have been extensively used in agriculture and in home pest control. However, the development of resistance in pests to these chemicals curtailed their commercial use (Hostetler and Brenner 1994). The molecular mechanism of action of pyrethroids was shown to be the delayed opening of the sodium channels in nerve membranes and alteration of calcium flux (Matsumura 1983; Narahashi 1986).

Azadirachtin, a natural compound, was isolated from seed kernels of neem trees (Butterworth and Morgan 1991). This compound was shown to exert a variety of biological effects on insects including lethality and growth regulation (Mordue and Blackwell 1993; Sayah et al. 1998; Mulla and Sue 1999). Neem-based insecticides are being investigated as alternatives to synthetic insecticides for the control of insect pests (Kreutzweiser 1997), especially where the development of resistance (Hostetler and Brenner 1994) is a major problem. Many of the natural products and synthetic insecticides used in the control of insect pests exhibit electrophysiological effects through interactions with receptors and ion channels (Scott et al. 1999).

Azadirachtin has been shown to alter the electrophysiological actions of insect chemosensory neruons while the cultured mammalian neurons were less sensitive (Scott et al. 1999). In this paper, we report the changes in electrophysiological properties of the giant interneurons in the abdominal ventral nerve cord (VNC) and the sensory neurons in the cercal nerves of the cockroach, *Peirplaneta americana* exposed to the synthetic pyrethroid, cypermethrin and/or the neem derivative, azadirachtin.

MATERIALS AND METHODS

Adult male cockroaches (*Periplaneta americana*) were acclimated to laboratory conditions (28^{0}C) for one week. Feeding was stopped one day before the experimentation to avoid any metabolic variations resulting from the diet. Only active and healthy looking animals were used for the electrophysiological studies. The insect was fixed to a wax-filled dissection board with the dorsal side up. The dorsal abdominal body wall was removed to expose the ventral nerve cord (VNC),

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terminal ganglion and cercal nerves. The VNC and cercal nerves, except when exposed to test solutions were always kept immersed in cockroach ringer (Vijayalakshmi et al. 1976) containing NaCl, 183 mM; KCl, 3 mM; $CaCl_2$, 1.5 mM; glucose, 57 mM; Na_2HPO_4, 0.43 mM; NaH_2PO_4, 0.18 mM, HEPES, 10 mM. Electrophysiological recordings were made at room temperature (28^0 - 30^0 C).

Spontaneous electrical activity (extracellular) was recorded using platinum electrodes from the nerve cord as well as the cercal nerves from control animals for 10 minutes to confirm whether there was sustained activity. Different concentrations (0.5 ppm, 1.0 ppm, 2.0 ppm, 4.0 ppm and 8.0 ppm) of cypermethrin (Northern Mineral Limited, India) and azadirachtin (Godrej, India) alone, and in combination (4 ppm azadirachtin; 4 ppm cypermethrin) were separately applied to the exposed nerve cord, or to cercal nerves in a Vaseline pool surrounding the tissue and allowed to perfuse for 10 minutes before the recordings were taken. To examine the giant interneruon responses in VNC, the cercal sensory hairs (sensilla) were stimulated mechanically with controlled air puffs (Edwards and Palka 1974), while the whole nerve was bathed in test solution or cockroach ringer.

The signals from VNC were amplified with a P5 Grass AC preamplifier and fed simultaneously to Systronics 5100 dual beam oscilloscope and tape recorder. A Grass C4 Kymograph camera was used to record the signals from the oscilloscope to photographic film. The tape-recorded information was fed to the 4-digit counter and oscilloscope to count the number of spikes/sec. The data were subsequently analyzed for statistical significance using Student's *t*-test.

RESULTS AND DISCUSSION

Azadirachtin produced an excitatory effect on the spontaneous electrical activity with an increase in both frequency and amplitude of spikes in the VNC of cockroach. Maximum excitatory effect was observed with azadirachtin at 4 ppm. However, the effect was significantly diminished at 8 ppm (Table 1). In addition to the spontaneous activity in the VNC and cercal nerves, the effect of azadirachtin (4 ppm) was also studied on air-evoked giant fibre responses 10 minutes after bath application. The air-evoked giant fibre responses and cercal nerve spontaneous activity showed moderate increases (Fig. 1).

A significant inhibition (17-64%) of VNC spontaneous activity was observed with cypermethrin in a concentration (0.5–4.0 ppm) dependent manner (Table 1). Giant fibre responses to the stimulation of cercal sensilla with air puffs also showed a marked reduction in the number of large amplitude spikes suggesting the inhibitory action of cypermethrin. The spontaneous activity in the cercal nerves also showed a similar effect providing supporting evidence for the observations made on the VNC activity (Fig. 2). The combined application of 4 ppm azadirchtin plus 4 ppm cypermethrin (synergist) produced interesting results. The elevatory action of azadirachtin on nerve activity in the cockroach was exacerbated by the inhibitory

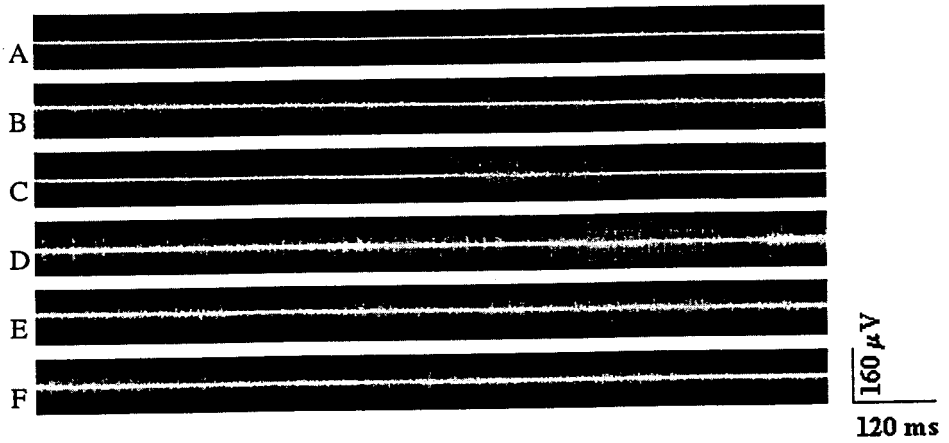

Figure 1. Azadirachtin induced changes in the electrical activity of cercal nerves and VNC of cockroach. Extracellular recordings were made from cercal nerves and VNC before and after perfusion with 4 ppm azadirachtin. A: Control activity from VNC. B: Increase in activity after perfusion with azadirachtin. C: Air-puff evoked giant interneuron responses (burst of high amplitude spikes). D: Increase in giant interneuron responses after perfusion with azadirachtin. E: Control cercal nerve activity. F: Increase in cercal nerve activity after perfusion with azadirachtin. Four separate animals were used for each set of recordings.

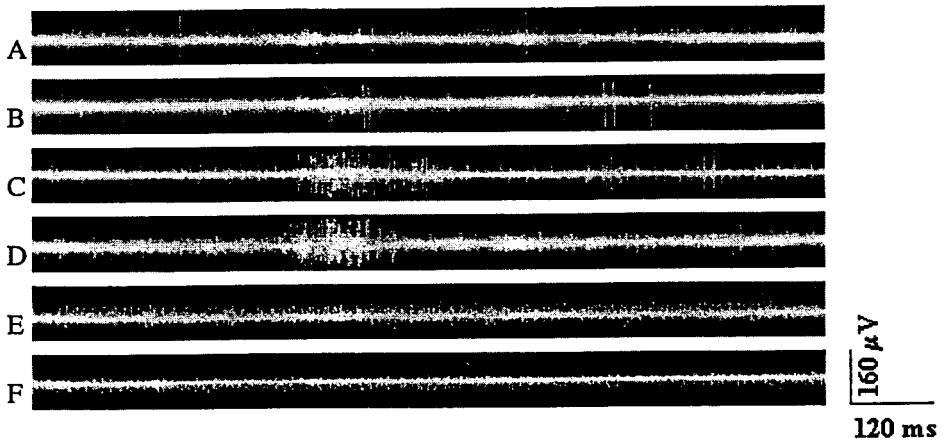

Figure 2. Cypermethrin induced changes in the electrical activity of cercal nerves and VNC of cockroach. Extracellular recordings were made from cercal nerves and VNC before and after perfusion with 4 ppm azadirachtin. A: Control activity from VNC. B: Decrease in activity after perfusion with azadirachtin. C: Air-puff evoked giant interneuron responses (burst of high amplitude spikes). D: Decrease in giant interneuron responses after perfusion with azadirachtin. E: Control cercal nerve activity. F: Decrease in cercal nerve activity after perfusion with azadirachtin. Four separate animals were used for each set of recordings.

Table 1. Changes in the Spontaneous electrical activity of the abdominal VNC of cockroach exposed to different concentrations of cypermethrin and/or azadirachtin. Electrical activity was presented as spikes/sec.

Conc. [ppm]	Control	Azadirachtin	Control	Cypermethrin	Control	Synergist
0.5	132 ±9	*149±11	162±6	134±9	173±13	*191±12
		(12.8)		(-17.3)		(10.4)
1.0	114 ±7	142±12	128±14	72±8	166±16	*142±9
		(24.0)		(-63.7)		(14.5)
2.0	126±8	195±13	170±16	76±7	138±9	82±7
		(54.8)		(-55.3)		(-40.5)
4.0	144±10	258±14	142±7	51±9	154±11	61±10
		(79.2)		(-64.0)		(-60.4)
8.0	135±11	178±9	155±10	54±8	152±9	51±7
		(31.8)		(-68.2)		(-66.4)

Each value is mean±S.D. of four separate recordings. Separate controls were used for each test solution and all the recordings were made between 09:00-11:00AM. The marginal variations in average control values shown above are because of animal to animal variation and the time of recording. Values in parenthesis show percent change from control. Values marked with asterisk (*) are not significant.

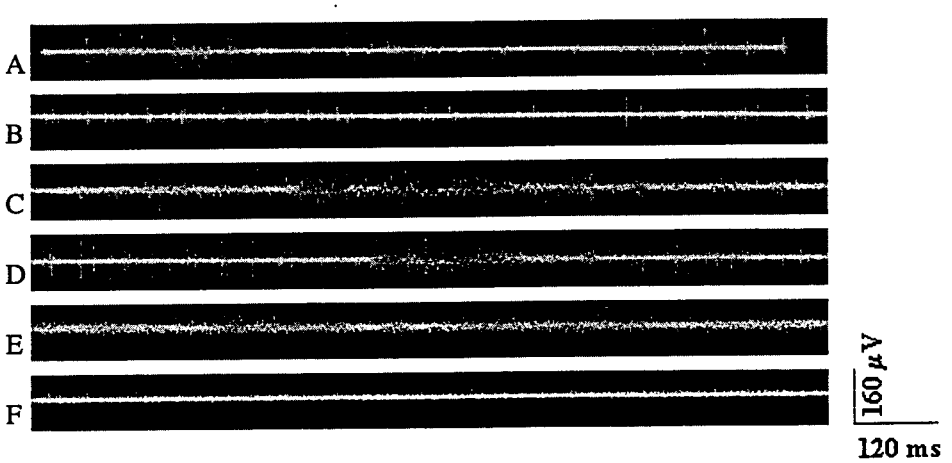

Figure 3. Synergist induced changes in the electrical activity of cercal nerves and VNC of cockroach. Extracellular recordings were made from cercal nerves and VNC before and after perfusion with 4 ppm synergist. A: Control activity from VNC. B: Decrease in activity after perfusion with synergist. C: Air-puff evoked giant interneuron responses (burst of high amplitude spikes). D: Decrease in giant interneuron responses after perfusion with synergist. E: Control cercal nerve activity. F: Decrease in cercal nerve activity after perfusion with synergist.

action of cypermethrin. As a result, the synergist compound, though producing a marginal increase in VNC spontaneous activity at 0.5 ppm exerted inhibitory action with subsequent increase in concentration from 1 to 8 ppm (Table 1). The synergist reduced the spontaneous activity of the VNC and cercal nerves as well as sensory mediated giant fibre nerve responses in the VNC, suggesting its inhibitions were (Fig. 3) similar to cypermethrin itself.

Extracellular application of azadirachtin produced significant neuronal excitability in the sensory and giant interneurons of the cockroach. Unlike the insect preparations, azadirachtin produced no effect on the resting potentials in cultured rat neurons but did alter the evoked action potentials (Scott et al. 1999). This effect was attributed to voltage activated K^+ currents. Perhaps azadirachtin is more effective in altering the electrical properties of intact nervous systems than cultured cell preparations.

Cypermethrin inhibited the spontaneous activity in the cercal nerves and VNC, and evoked potentials in the giant axons of the cockroach. The mechanism of toxicity of pyrethroids has been shown to include interference with the sodium gate in nerve membranes in insects (Narahashi 1962). Inhibition in Ca^{2+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase activity has also been reported in the CNS of pyrethroid-exposed insects (Reddy et al. 1994). The observed neural action of cypermethrin in the cockroach could also result from interference with calcium regulation (Matsumura 1983). The decrease in the amplitude and frequency of giant fibre responses to the air-puff stimuli applied to the cercal sensilla can partially be attributed to the damage caused to the cercal nerves exposed to cypermethrin. Many of the natural products and synthetic insecticides used in the control of insect pests exert electrophysiological effects through interactions with receptors and ion channels leading to a variety of responses including death (Scott et al. 1999). It is suggested that cypermethrin and azadiachtin interfere with ion channels and together form a novel insecticide with little or no development of resistance. However, further studies in this direction need to be performed to confirm this possibility.

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