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Bioelectrical Activity of Isolated Ventral Nerve Cords of the Cockroach, *Periplaneta americana* L., Treated with Toxaphene

C. C. Dary and L. A. Crowder
Department of Entomology, University of Arizona, Tucson, Ariz. 85721

The mode of action of toxaphene ($C_{10}H_{10}Cl_8$, chlorinated camphene containing 67-69% chlorine) is not known. Although it is generally accepted that this insecticide acts on the nervous system, a precise locus of action has not been defined. LALONDE and BROWN (1954) observed the action of toxaphene on sensory nerves of Periplaneta americana (L.) isolated from the central nervous system. Following a latent period of 3 h, toxaphene generated 100 μv spikes at a frequency of 180 spikes/sec for 30 min, after which all activity ceased. In a study with Blattella germanica (L.), WANG and MATSUMURA (1970) observed toxaphene neurotoxicity after a 14 min latent period. Toxaphene appeared to be more highly neurotoxic (threshold concentration 6 x 10^{-7}M) than was indicated by in vivo toxicity.

The objective of this investigation was to examine the action of toxaphene on activity of the ventral nerve cord (VNC) in P. americana. The results could aid in elucidating the mode of action of toxaphene.

METHODS AND MATERIALS

Technical grade toxaphene was obtained from Hercules, Inc. (batch sample X16189-49). Solutions in mineral oil were prepared serially from a stock solution of 10^{-1}M toxaphene in acetone.

Adult males of P. americana were maintained in a rearing room (22-23°C, 70% RH, and 12:12 light-dark cycle), on a mixture of honey, glycerine, and Purina^R Dry Dog Chow (2:2:6, v/v) and an ample water supply. For electrophysiological studies, the head, legs, and wings were removed and a mid-dorsal incision made the entire length of the body. VNC (2nd through 6th ganglia inclusive) were removed, promptly cleared of fat body and tracheae, and placed in saline (YAMASAKI and NARAHASHI 1959).

An extracellular suction electrode system was used to record from VNC (FLOREY and KRIEBAL 1966). Glass capillary tubes, fashioned to approximate the outer diameter of a length of VNC, were used to achieve contact with a Ag-AgCl electrode through a tight junction with intracapillary saline. A Ag-AgCl rod, 0.5 mm in diameter and 3.0 cm long, was used as the indifferent electrode. VNC were bathed in saline (pH 7.1, 22-24°C) perfused through a 4 x 6 x 2 cm plexi-glass well at the rate of 1.0 ml/min.

Nerve activity was observed on a Tektronix 5103 dual beam

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storage oscilloscope amplified by a Grass P-5 amplifier. Frequency and bursts of spikes were measured using a Tektronix DC-502 frequency counter. Spike and burst intervals were measured on a Mentor N-750 spike analyzer. Oscillographs were taken of activity periods throughout the testing procedure.

Specimens were observed for spontaneous activity before pharmacological tests were made. Twenty untreated controls and 20 mineral oil controls were observed for up to 8 h to clearly distinguish effects generated by preparation from those induced by mineral oil and toxaphene. Mineral oil and toxaphene (0.21 µg to 2.07 mg) were introduced by injecting the dose in 0.05 cc mineral oil in close proximity to VNC.

Toxaphene was tested in a stepwise manner, beginning with lowest concentrations first. Observations of activity were made for at least 2 h with each concentration (20 VNC/concentration). VNC which demonstrated a response to a particular concentration were studied until activity ceased. The effects of toxaphene on VNC were analyzed by comparing controls with various concentration levels of toxaphene. Latent periods, i.e., times between the introduction of toxaphene and the onset of neural response, together with spike frequency and amplitude generated during particular phases of the testing period, were used as criteria for analysis.

RESULTS AND DISCUSSION

Toxaphene was found to be moderately toxic to P. americana at 24 hr; the ED-50 and LD-50 were 57.3 µg/g and 124.4 µg/g, respectively. COCHRAN (1955) found the LD-50 at 96 h to be 30 and 70 µg/g for males and females, respectively.

Symptoms of poisoning much like that observed by HARVEY and BROWN (1951) became apparent within 24 h after injection. The syndrome of toxicity was divided into 3 periods: (1) prostration with leg movements, (2) prostration, and (3) moribundity. Insects recorded as displaying symptom 1 demonstrated tactile sensitivity, partial paralysis, and excitation which often caused them to fall on their dorsal surface and vigorously twitch their legs. Symptom 2 was recorded for insects displaying slight tremors of the labial palps, antennae, and abdomen while on their dorsum. Electrophysiological recordings from VNC of these insects showed endogenous activity (Fig. 1). Cockroaches that appeared to be dead were recorded as moribund, symptom 3. They did not respond to tactile stimulation, and heart rate was either slowed and irregular or there was total absence of heart activity. Nerve transmission could, however, still be observed (Fig. 2).

There were 2 periods of endogenous or spontaneous activity observed with VNC of P. americana controls. Period 1 was characterized by strong activity which followed immediately after VNC contact with the electrode and lasted an average of 4.5 min. This vigorous activity was observed for all VNC, both control and toxaphene-treated, and consisted of spike bursts or trains of high amplitude spikes generated at an average frequency of

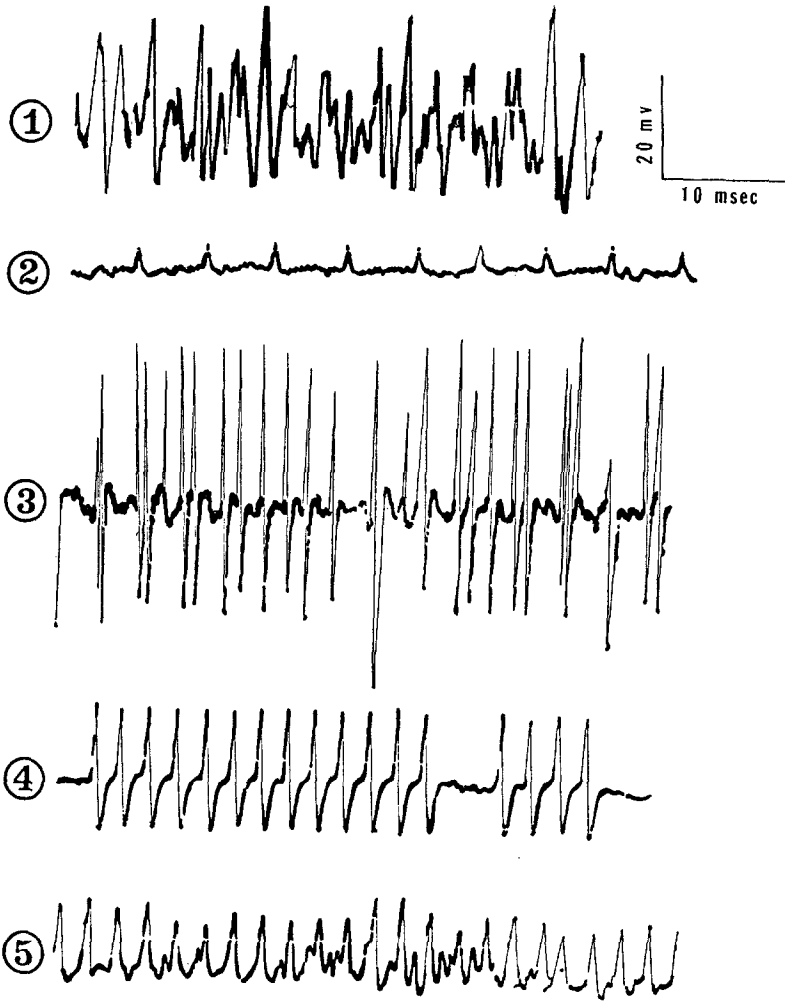

Fig. 1-5. Representative oscillographs of bioelectrical activity observed during treatment of ventral nerve cords (2nd-6th ganglia inclusive) of P. americana to toxaphene. 1, Removed from insect displaying symptom 2 of poisoning. 2, Removed from insect displaying symptom 3 of poisoning. 3, Intense activity of isolated VNC. 4, Bursts or trains of spikes following intense activity (burst interlude). 5, Irregular spike discharge following burst interlude.

27.1 Hz. The first period of activity appeared to be a form of dissection or adjustment shock. Discharge was intense, but did not last much over 4 min. This activity might be a function of adjustment to temperature, pH, or ion concentration of the bathing saline. It might also be due to the pressure placed on the tissue during the initial drawing of the VNC into the orifice of the suction electrode. In either event, this activity subsided and was not observed again over the complete period of approximately 8 h with the controls.

The vigorous form of endogenous activity gave way to that observed in period 2, consisting of variable nerve activity and lasting about 3 h. Single spikes of variable amplitudes and frequencies, and bursts or trains of spikes of constant amplitudes and frequencies were observed throughout the period.

Approximately 3 h after VNC-electrode contact, activity was absent. Controls studied for strictly endogenous activity showed that there was no resumption of period 2 type activity during the next 5 h. Mineral oil did not appear to have an effect on VNC activity unless it was tested prematurely, before the end of period 2. In that case, activity much like that observed during period 1 was generated. Controls tested with mineral oil between 3 h to 4 h 45 min after VNC-electrode contact did not show any increase in activity. Pharmacological testing began with VNC at the end of period 2.

Toxaphene generated activity in VNC. The most evident demonstration of neural sensitivity was a display of intense activity shortly after introduction of toxaphene (Fig. 3). This intense activity was characterized by spikes of varying amplitudes generated at high frequencies (70.5 to 159.4 Hz). It started with a range of low amplitude spikes (4.7 to 51.7 mv) occurring at 2.3 to 116.9 Hz. Spike amplitude and frequency increased rapidly. Nerve activity attained highest levels as shown in Fig. 3, and then after 1 to 5 min, was absent. No difference could be detected between the intense activity generated by doses ranging from 0.21 µg to 2.07 mg.

Duration of the latent period between toxaphene introduction and the first signs of effects were influenced by concentration (Table 1). Intense activity was generated an average of 22.6 min after introduction of 0.21 µg toxaphene. The latent period decreased as the concentration increased; with 2.07 mg toxaphene, the latent period was only 1 min.

After intense activity subsided, bursts of spikes uniform in amplitude were observed (Fig. 4), at the rate of 7/min with a mean spike frequency of 27.5 Hz. This activity (burst interlude) continued for approximately 30 min; it was followed by the absence of activity in some VNC and irregular activity (spikes of varied amplitudes and frequencies) in others (Fig. 5). Concentration of toxaphene was not related to duration of the burst interlude.

Toxaphene generated activity in isolated VNC at concentration levels which were consistent with *in vivo* toxicity. The latent

TABLE 1.

Measurements of bioelectrical activity from isolated ventral nerve cords (2nd-6th ganglia inclusive) of the cockroach, P. americana, exposed to toxaphene injected into the bathing solution.

Dose	Stage of Activity	Duration Time (min)	Spike Amplitude (mv)	Spike Frequency (Hz)
0.21 µg	Latent Period[b]	22.6		
	Intense Activity[c]	2.4	146.8	70.5
2.07 µg	Latent Period	10.8		
	Intense Activity	1.8	70.5	159.4
20.7 µg	Latent Period	8.7		
	Intense Activity	1.3	131.0	42.3
207.3 µg	Latent Period	1.5		
	Intense Activity	0.8	94.0	149.0
2.07 mg	Latent Period	1.0		
	Intense Activity	4.7	70.0	138.5

[a] Means of 20 preparations/dose.

[b] Defined as stage between introduction of toxaphene and the onset of nerve discharge.

[c] Characterized by spikes of varying amplitudes generated at high frequencies (70.5 to 159.4 H).

period between introduction of toxaphene and neuroactivity in isolated VNC varied with concentration. This evidence indicates a site of action in the nervous system, consistent with the observations of LALONDE and BROWN (1954) for the cyclodienes, and WANG and MATSUMURA (1970) for toxaphene and the cyclodienes.

SUMMARY

Effects of toxaphene were studied with the ventral nerve cord (VNC) of the cockroach, Periplaneta americana (L). Intense forms of activity were observed in isolated nerve preparations treated with toxaphene. Latent periods between introduction of toxaphene and onset of intense activity decreased as the concentrations were increased.

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