

# Wasp Venom Blocks Central Cholinergic Synapses to Induce Transient Paralysis in Cockroach Prey

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Received 12 August 2002; accepted 7 October 2002

**ABSTRACT:** The parasitoid wasp *Ampulex compressa* induces a set of unique behavioral effects upon stinging its prey, the cockroach. It stings into the first thoracic segment inducing 2 to 3 min of transient flaccid paralysis of the front legs. This facilitates a second sting in the cockroach's head that induces 30 min of excessive grooming followed by a 2 to 5-week long lethargic state. In the present study, we examine the immediate effect of the first sting, which is a transient paralysis of the front legs. Using radiolabeled wasps, we demonstrate that the wasp injects its venom directly into the cockroach's first thoracic ganglion. The artificial injection of milked venom into a thoracic ganglion abolishes spontaneous and evoked responses of the motoneurons associated with leg movements. To investigate the physiological mechanism of action of the venom, we injected venom into the last abdominal ganglion of the cockroach, which

houses a well-characterized cholinergic synapse. Injected venom abolishes both sensory-evoked and agonist-evoked postsynaptic potentials recorded in the postsynaptic neuron for 2 to 3 min without affecting action potential propagation. Thus, the venom blocking effect has a postsynaptic component that follows the same time course as the transient paralysis induced by the thoracic sting. Finally, injection of a nicotinic antagonist in the front thoracic ganglion induces paralysis of the front legs. We conclude that the transient paralytic effect of the thoracic sting can be mainly accounted for by the presence of a venom active component that induces a postsynaptic block of central cholinergic synaptic transmission. © 2003 Wiley Periodicals, Inc. *J Neurobiol* 54: 628–637, 2003

**Keywords:** wasp; *Ampulex compressa*; cockroach; venom; nicotinic receptor

## INTRODUCTION

Venomous predators manufacture highly specific neurotoxins to incapacitate their prey. In most known cases, the ultimate physiologic effect of such neurotoxins is to prevent the prey from escaping or fighting. A common strategy applied by most venomous animals is to inject their venom into the prey's circulation system to cause muscular paralysis (for reviews, see Rathmayer, 1978; Piek and Spanjer, 1986; Piek,

1990; Adams, 1996). In most wasp species studied so far, the venom is injected into the hemolymph of the prey and diffuses to the periphery to interfere with the release of excitatory transmitter at the neuromuscular junctions (Beard, 1952; Piek, 1982; Piek and Spanjer, 1986; Eldefrawi et al., 1988; Piek, 1990). The impairment of neuromuscular transmission results in a muscular paralysis of the prey.

*Ampulex compressa*, a parasitoid solitary wasp, applies a different strategy when it hunts the cockroach, *Periplaneta americana*, to serve as a food supply for its larvae (Williams, 1942). The cockroach is not paralyzed, but rendered helplessly submissive after the wasp stings into its head to cause 30 min of excessive grooming (Weisel-Eichler et al., 1999) followed by a 2- to 5-week long lethargic state (Fouad et

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Contract grant sponsor: BSF; contract grant number: 2001044.

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al., 1994, 1996; Libersat et al., 1999). In this lethargic state, the cockroach is unresponsive, and does not initiate movement (Fouad et al., 1994, 1996; Libersat et al., 1999). It is docilely led by the wasp to a burrow to be later consumed alive by its larvae.

The sting in the head, which lasts more than 1 min, is preceded by a brief (10–20 s) sting in the first thoracic segment housing the first thoracic ganglion. This ganglion contains the motor circuitry responsible for generating front leg movement (Fourtner and Kaars, 1990). Cockroaches stung only once, in the prothorax, exhibit flaccid paralysis of the front legs from which they recover within a few minutes (Fouad et al., 1994). Because this first sting prevents the cockroach from using its forelegs to fight off the wasp, it presumably facilitates the delicate sting into the head of the cockroach.

Our goal in the present study was to explore the mechanism of action of the venom for the thoracic sting, which causes the transient flaccid paralysis of the front legs. First, we wished to determine whether the wasp injects its venom into the first thoracic ganglion or in its vicinity. Next, we explored the physiologic mechanisms of action of the venom to induce the transient flaccid paralysis of the front legs. Because most sensory input pathways as well as many central synapses in the cockroach use acetylcholine as the excitatory transmitter (Sattelle, 1985; Gundelfinger, 1992), we used a well-characterized cholinergic synapse to investigate the possibility that the venom may interfere with central cholinergic neurotransmission to induce the front leg paralysis.

## METHODS

### Animals

The wasps, *Ampulex compressa* Fabricius (Hymenoptera: Sphecidae), were raised at 30°C and 50% humidity on a 12L:12D cycle in Perspex cages provided with abundant water and honey. Adult cockroaches (*Periplaneta americana*) were raised at 25–30°C in plastic cages and provided with abundant water and cat food pellets. All electrophysiologic experiments were performed on adult male cockroaches.

### Radiolabeling of Venom

The venom glands of three wasps were first depleted by milking. The wasps were then injected with a mixture of <sup>14</sup>C radiolabeled amino acids (Sigma, 10 μCi per wasp). Two days later, the venom of these wasps was milked and separated by SDS-PAGE. The peptides were stained with Coomassie blue and then exposed for 5 weeks to X-ray film.

Five more wasps, radiolabeled in the same manner, were allowed to freely sting several cockroaches 2 days after the injection of radiolabeled amino acids. To check the possible diffusion of labeled venom from the surrounding thoracic tissue into the CNS, we injected cockroaches with a mixture of radiolabeled amino acids outside of the central nervous system and followed the distribution of radioactive signal. The stung and injected cockroaches were collected immediately after the sting and put on ice to reduce diffusion.

### Liquid Scintillation

The amount of radioactivity in the tissues of cockroaches stung by the labeled wasps ( $n = 15$ ) or injected with radioactive amino acids ( $n = 15$ ) was assessed using liquid scintillation (Ultima Gold LSC cocktail and Liquid Scintillation Analyzer 2100TR, Packard). We examined the first, second, and third thoracic ganglia (T1, T2, T3) and the surrounding non-neuronal tissues.

### Venom Milking

Wasps were immobilized with CO<sub>2</sub> and confined to a small, conical, plastic tube open at both ends. A modified syringe plunger was fit to one end of the tube and used to provoke the wasp to sting a small piece of Parafilm (American National Can) held in front of the other end. Venom droplets were collected into about 10 μL/wasp of 10 mM HEPES buffer (pH 7.4 with NaOH) containing 0.1 mM PMSF, a Serine protease inhibitor (Sigma). Venom collected from 15 to 20 wasps was dried by lyophilization and kept at –80°C.

### Injections

Venom and d-tubocurarine (d-tubocurarine chloride; Sigma, St. Louis, MO) were dissolved in cockroach saline (composition in mM: NaCl 214, KCl 3.1, CaCl<sub>2</sub> 9, Sucrose 50, HEPES 5, pH 7.2 and 0.1 % Janus green to visualize the injection) and injected at quanta of 23 nanoliters with a nano-volumetric injector (Medical systems Ltd, Greenvale, NY). The amount of venom in this volume roughly matches that of a single injection by an individual wasp during milking. The sheath covering the ganglion was partially removed with fine forceps to facilitate the penetration of the glass pipette of the injector. Injections were confirmed visually through the microscope.

### Electrophysiology

**Thoracic Ganglion Preparation.** Adult cockroaches were cooled-anesthetized (2°C) and pinned ventral side up on a Peltier device; a flap was opened in the cuticle to expose the second thoracic ganglion (T2) and the associated motor nerves. Extracellular recordings of action potentials were monitored by a pair of hook electrodes placed around branch 5r1 of nerve 5. This branch contains only five axons (Pearson and Iles, 1971), the axons of the fast and slow

excitatory and three inhibitory motoneurons to the coxal muscle. The giant axons of the ventral nerve cord were stimulated with a train of pulses (20 pulses, pulse duration 0.5 ms at a period of 1.5 ms) every 10 s, to elicit a few spikes in the motoneurons recorded in nerve 5r1.

**Abdominal Ganglion Preparation.** Adult cockroaches were anesthetized with CO<sub>2</sub> and pinned dorsal side up on a recording platform after ablating the legs and wings. The dorsal abdominal cuticle was removed and the nervous system was exposed from the first abdominal ganglion (A1) to the most posterior ganglion (A6). The preparation, which included the cerci and the abdominal portion of the nerve cord from A1 to A6, was dissected out and pinned on a dish coated with Sylgard (Dow-Corning, Midland, MI). Preparations were bathed in saline (composition in mM: 214 NaCl, 3.1 KCl, 9 CaCl<sub>2</sub>, 50 sucrose, 10 TES; pH 7.2) at room temperature. The activities of the giant interneurons (GIs) and cercal sensory neurons were recorded extracellularly with monopolar stainless steel electrodes placed next to the nerve cord and cercal nerve respectively and isolated with Vaseline. These electrodes were also used to stimulate the nerves in some of the experiments. The last abdominal ganglion was desheathed to expose the cell bodies of the GIs. A single GI3 was recorded in the soma using a glass microelectrode filled with 6% Carboxyfluorescein in 1 M KOH. The recorded GI3 was filled with Carboxyfluorescein and identified visually under an Olympus compound fluorescent microscope after each experiment.

Tactile stimuli were applied to one of the cercus with the end of a small plastic rod connected to a loudspeaker driven by a pulse generator. Wind stimuli were produced by a loudspeaker connected to a tube via a funnel as described in Fouad et al. (1994). Ionophoresis of CCh [Charbamilcholine chloride (Charbachol), Sigma, St. Louis, MO] was done using glass pipettes similar to those used for the intracellular recordings. The pipette was filled with 1 M solution of CCh dissolved in distilled water. A negative holding current of -15 nA was used to prevent leaking of CCh and a positive current of 10–15 nA was injected for 500 ms every 15 s. The ionophoresis pipette was positioned in the neuropile in the posterior part of the ganglion next to the estimated dendritic field of the recorded GI, which is contralateral to the recording electrode. The current injection was then incremented until a PSP of maximal amplitude was reached.

In those experiments where the effect of venom on action potential propagation was investigated, action potentials were elicited in the axons of the GIs between A4–A5 connectives via a monopolar, extracellular electrode and monitored with another electrode placed on the A3–A2 connectives. Venom was then injected into the A3 ganglion between the stimulating and recording electrodes.

## Data Analysis

Neuronal signals were recorded on videotape (Neuro-corder DR-890, Neurodata Instrument Corp., New York, NY) and digitized with a NB-MIO-16 analog-to-digital board (Na-

tional Instruments, Austin, TX). Data were recorded and analyzed off-line using a custom-made data analysis program. Multiple replicates (at least  $n = 5$ ) were taken from each experiment. The postsynaptic potential (PSP) amplitude was measured with a cursor function available in the data acquisition/analysis software. Mean number of spikes and other statistical parameters were calculated with Excel (Microsoft, Redmond, WA). ANOVA was used for comparing the scintillation counts and the Mann-Whitney *U*-test was used for comparing the standardized number of spikes of venom or saline injected preparations (Systat and SigmaStat). Data are presented as mean ( $\pm$ S.E.).

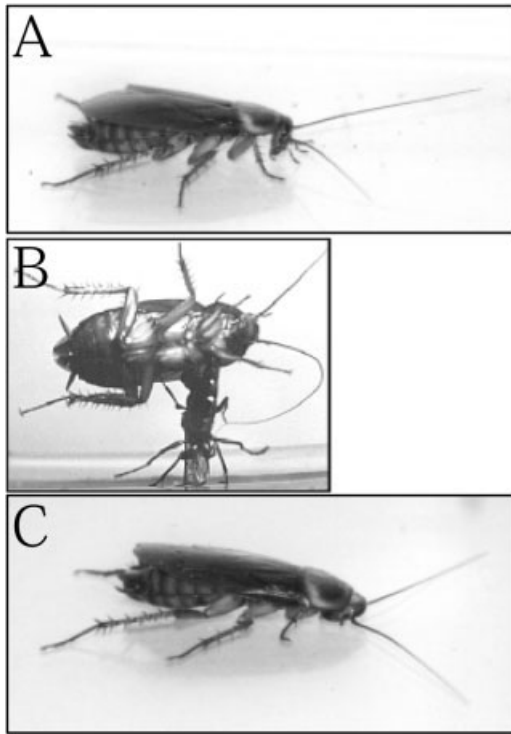
## RESULTS

### Behavioral Effect of the Thoracic Sting

The wasp stings the cockroach first in the thorax and then in the head. The first sting, which lasts 10–20 s, enters through the soft membrane between the front leg and the prothorax, and seems to be directed towards the first thoracic ganglion. Immediately after the sting, the cockroach shows a flaccid paralysis of the front legs resulting in a dramatic postural change and a typical “head-down” posture [Fig. 1(C)] that lasts 3 to 5 min. If the wasp is allowed to apply only the first sting in the cockroach thorax (or even multiple thoracic stings) only the transient paralysis, but no other effect, takes place.

### Site of Injection of the Venom

To determine whether or not the wasp stings directly in the thoracic ganglion, we labeled venom peptides *in vivo* by injecting radioactive amino acids into wasps, allowed them to sting cockroaches, and then measured radioactivity levels in specific tissues of the stung cockroaches. To verify the incorporation of radiolabeled amino acids, the venom of three radiolabeled wasps was milked 2 days after the injection of radiolabeled amino acids and separated by SDS-PAGE. The gel was Coomassie-stained and exposed to X-ray film for 5 weeks. The milked venom resolved on SDS-PAGE contained more than 10 peptides, all of which were labeled to various extents [Fig. 2(A)]. Five other wasps were allowed to sting cockroaches 2 days after the injection of radiolabeled amino acids. The level of detected radioactivity [Fig. 2(B)] was always significantly higher ( $p < .01$ , ANOVA between tissues) in the first ganglion (T1,  $71 \pm 15\%$ ) than in the second (T2,  $18 \pm 17\%$ ) and third (T3,  $1 \pm 1\%$ ) thoracic ganglia and the non-neuronal tissue ( $9 \pm 5\%$ ) in each stung cockroach. To check the alternative possibility of diffusion of the venom from the



**Figure 1** *Ampulex compressa* injects its venom into the thorax of a cockroach to induce a transient paralysis of the front legs. (A) A typical upright posture of the cockroach before the thoracic sting. (B) The wasp delivers the thoracic sting through the soft cuticle between a front leg and the prothorax. (C) A few seconds after the sting, the cockroach's front legs are flaccidly paralyzed and it cannot support its own weight.

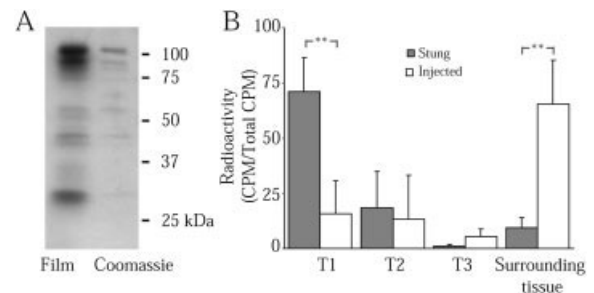
surrounding tissue into the nearby nervous tissue, we injected radiolabeled amino acids into the thorax outside of the ventral nerve cord of cockroaches and measured the radioactive level in the same tissues as in the stung cockroaches. In the injected cockroaches, most of the radioactive signal was detected in the surrounding thoracic non-neuronal tissue ( $65 \pm 20\%$ ) while significantly less ( $p < .01$ ) was found in the thoracic ganglia (T1,  $16 \pm 15\%$ ; T2,  $13 \pm 20\%$ ; T3,  $6 \pm 3\%$ ). Thus, we conclude that the high radiolabeling in the first thoracic ganglion of cockroaches stung by radiolabeled wasps is not due to diffusion from the surrounding tissues. Rather, the wasp penetrates the soft cuticle of the leg joint with its sting and injects its venom *in situ* directly into the first thoracic ganglion.

### Effect of Venom on Motor Output in the Thorax

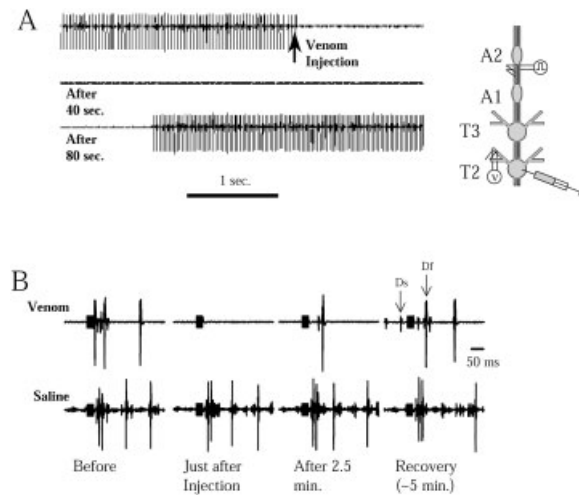
Once we established that the venom is injected into the thoracic ganglion, we wished to reproduce the

effect of the wasp sting on the cockroach forelegs motor activity with a direct injection of venom into one of the thoracic ganglion. To this goal, we milked wasps to collect venom and injected it into a thoracic ganglion while recording from branch 5r1 of nerve 5. This branch contains only five axons: a fast motoneuron (Df), a slow motoneuron (Ds), and three inhibitors (Pearson and Iles, 1971). In most preparations, Ds fired spontaneously before the injection at an average rate of 33.3 Hz (range 9.3–85.7 Hz) [Fig. 3(A)]. While the injection of saline had no significant effect on the firing rate of this motoneuron ( $n = 7$ ), upon injection of venom, the tonic firing of Ds ceased completely within seconds ( $n = 7$ ) and started to recover within 30 to 90 s. After this period, in five of seven preparations, the firing rate was 72% higher on average than before the venom application. In the remaining two preparations, the firing rate returned to a lower value than before the venom injection (52% less, on average).

In another set of experiments, prior to the venom injection, axons of the ventral nerve cord were stimulated with a short train of stimuli every 10 s, to elicit several spikes in the fast and slow motoneurons recorded in nerve 5r1. In all trials, the evoked motoneuron response was abolished within seconds following the injection of venom ( $n = 12$ ) but was unaffected by



**Figure 2** Radiolabeled venom is localized to the first thoracic ganglion. (A) Venom, milked from wasps that were injected with radiolabeled amino acids, as visualized with Coomassie stain on SDS-PAGE (right lane) and exposed to X-ray film (left lane). The venom contains more than 10 peptides, all of which are radiolabeled to a different extent. (B) In cockroaches stung by radiolabeled wasps, most of the radioactive signal is detected in the first thoracic ganglion (T1). The remaining radioactivity is detected in the second (T2) and third (T3) thoracic ganglia and in the surrounding, non-neuronal, tissue. In cockroaches injected with radioactive amino acids, most of the radioactivity is detected in the surrounding tissue while the rest of the radioactivity is detected in the thoracic ganglia. A significant ( $p < .01$ ) difference in radioactivity levels is found between stung and injected cockroaches only in T1 and in the surrounding tissue.



**Figure 3** Venom injected into a thoracic ganglion blocks spontaneous and evoked motor activity. (A) Extracellular recording of motor nerve 5r1 (diagram). In most preparations, the slow motoneuron (Ds) fires spontaneously. The injection of venom ( $n = 7$ ) abolishes it within seconds. The tonic activity recovers spontaneously within 30 to 90 s. (B) The ventral nerve cord is stimulated with a train of pulses to evoke a response of the fast (Df) and slow (Ds) motoneurons (diagram). This response is blocked within seconds after venom injection ( $n = 12$ , top traces) and recovers within a few minutes. The injection of saline has no effect ( $n = 9$ , bottom traces).

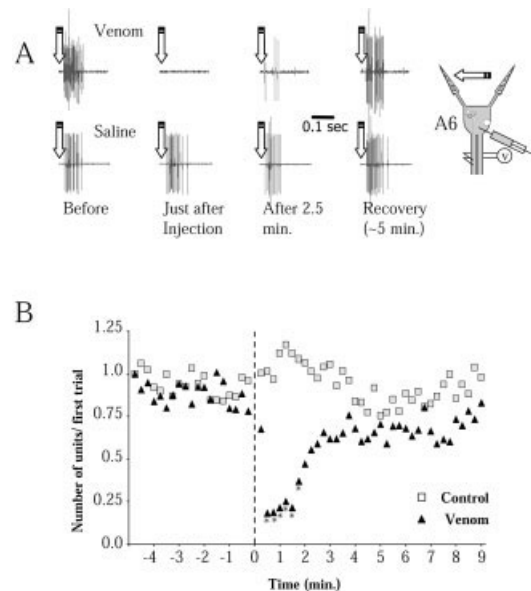
the injection of saline ( $n = 9$ ). The response began to recover within 45 to 115 s [Fig. 3(B)].

### Cellular Mechanisms of Action of the Venom

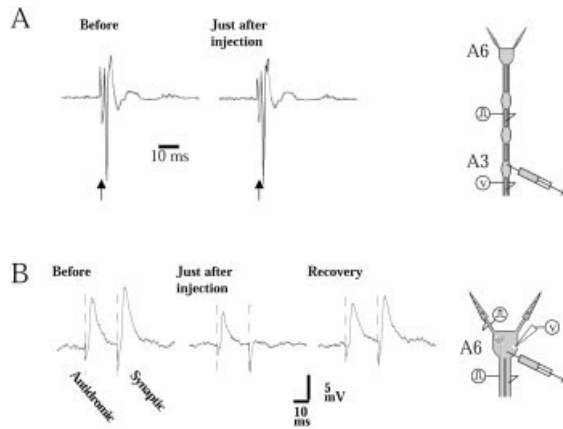
To examine the effect of venom on synaptic transmission and possibly on action potential generation and propagation, we turned to the cercal circuitry in the last abdominal ganglion (A6) of the cockroach's CNS, as a model. The sensory-to-giant interneuron (GI) synaptic connection in A6 is a well-characterized cholinergic synapse (Sattelle et al., 1976; Callec et al., 1982; Blagburn et al., 1985; Blagburn and Sattelle, 1987). To establish the sensory to GI synapse as a model for the venom effect on thoracic transmission, we microinjected venom into A6 while measuring the wind-evoked response of the GIs (Fig. 4, diagram). A wind sensory stimulus applied to the cerci evokes a reliable and steady burst of action potentials in the GIs [Fig. 4(A)]. Within seconds after the injection of venom, the evoked response in the GIs decreased by  $80 \pm 22\%$  (and in most cases was completely abolished) for roughly two minutes [Fig. 4(B)]. After 2 min, the evoked response started to recover gradually

and fully after 3 min ( $p < .05$ , Mann-Whitney  $U$ ). Injection of saline in A6 had no significant effect on the evoked response of the GIs [Fig. 4(A) and (B)].

Two possible mechanisms could, in theory, account for the absence of response in the GIs immediately following the venom injection. The venom injected in A6 could either block the initiation and propagation of the action potential in the GI or block the cholinergic synaptic transmission between sensory neurons and the GI. To examine whether the venom interferes with the propagation of the action potential, we first elicited action potentials in the nerve cord axons between abdominal ganglia A5 and A4 [Fig. 5(A), diagram]. We then injected venom into the abdominal ganglion A3 while recording the propagating action potentials beyond this ganglion, between abdominal ganglia A3 and A2. The injection of venom did not prevent the propagation of action potentials through the ganglion [Fig. 5(A),  $n = 5$ ]. As a



**Figure 4** Venom blocks the sensory evoked response of giant interneurons in the last abdominal ganglion (A6). (A) A sensory stimulation (arrow) is applied to the cerci while the evoked response of the giant interneurons (GIs) is recorded extracellularly on the nerve cord (diagram). Each sensory stimulus produces a reliable burst of action potentials in the GIs. This evoked response is abolished within seconds after injection of venom and then gradually recovers within minutes (top traces). The injection of saline has no effect (bottom traces). (B) Each data point represents the number of units in a given trial normalized to the first trial. The evoked response decreases significantly (Mann-Whitney  $U$ -test,  $*p < .05$ ) by more than 80% on average upon injection of venom ( $n = 5$ ). The response then gradually recovers 3 min after the injection of venom. The injection of saline has no effect on the evoked response.



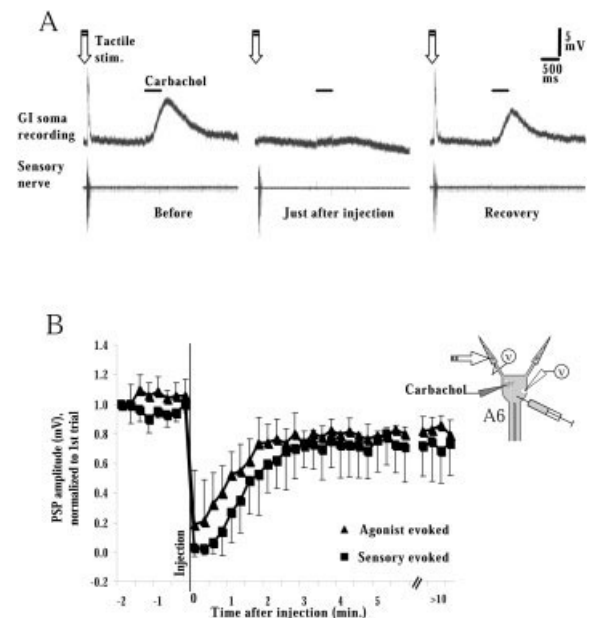
**Figure 5** Venom does not block action potential propagation. (A) The axons of GIs are recruited with an electrical stimulus (arrow) applied to the nerve cord via an electrode and their propagation through abdominal ganglion A3 is monitored with another electrode on the nerve cord (diagram). The average traces of 15 evoked compound action potentials from a typical experiment are represented. Venom injected into abdominal ganglion A3 does not prevent the propagation of action potentials through this ganglion ( $n = 5$ ). (B) Two consecutive action potentials are induced 25 ms apart. The first is induced by direct antidromic stimulation of the nerve cord and the second by mechanical stimulation of cercal sensory receptors, which activate the sensory to GI synapse (diagram). Only the synaptically evoked action potential is completely eliminated by a venom injection into the last abdominal ganglion (A6) (middle trace,  $n = 5$ ) and recovers within 2–5 min while the antidromically evoked action potential is not blocked.

positive control, we confirmed the potency of the venom by injecting it into A6, where it blocked the GIs response (not shown).

To further test the effect of the venom on action potential propagation and on synaptic transmission, we used a different experimental protocol in which we intracellularly recorded from a GI soma and alternately stimulated the ventral nerve cord and the cercal nerve with extracellular electrodes [Fig. 5(B), diagram]. Using this sequential stimulation protocol, we could produce an antidromic action potential in the GI's axon followed by a synaptically mediated action potential. If the venom affects mainly synaptic transmission, one would expect it to block only the synaptically mediated action potential and leave the antidromic action potential unaffected. However, if the venom also affects action potential propagation, one would expect it to block both the synaptically mediated and the antidromic action potential. In fact, injection of venom blocked only the synaptically mediated action potential and does not prevent the

propagation of the antidromic action potential [Fig. 5(B)]. Therefore, we conclude that a block of the sensory to GIs synaptic connection can account for the block of the evoked response in the GIs, with little effect on the generation and propagation of the action potential.

To characterize the effect of the venom on cholinergic synaptic transmission, we intracellularly recorded from a GI soma (Fig. 6, diagram) while alternately inducing an excitatory postsynaptic potential (PSP) by mechanical stimulation of the cercal receptors and by direct ionophoresis of a nicotinic agonist, Carbachol (CCh) (Blagburn and Sattelle, 1987). With this experimental protocol, we could evaluate the contribution of presynaptic versus postsynaptic components to the block of synaptic transmission. If the venom exerts a presynaptic effect, one would expect it to block only the sensory evoked PSP and leave the



**Figure 6** Venom blocks cholinergic synaptic transmission. (A) Two consecutive postsynaptic potentials (PSPs) are evoked 2 s apart (top trace). The first PSP is evoked by mechanical stimulation of cercal sensory receptors (arrow). The second and slower PSP is evoked by direct application of the nicotinic agonist Carbachol. The response of the cercal sensory neurons is also monitored (bottom trace). Both PSPs, recorded in the GI soma, are abolished within seconds of the injection of venom into ganglion A6 and then both PSPs gradually recover ( $n = 5$ ). (B) Each data point represents the PSP amplitude in a given trial, normalized to the PSP amplitude of the first trial. Agonist and sensory evoked PSPs show a similar time course of blocking and recovery. Both sensory evoked (squares) and agonist evoked (triangles) PSPs are abolished within seconds after venom injection and gradually recover within 2–3 min.

agonist evoked PSP unaffected. Conversely, if the venom exerts a postsynaptic effect, it should block both PSPs. This second alternative does not rule out a possible presynaptic effect as well. In fact, injection of venom abolished both the sensory and agonist evoked PSPs. This indicates that the block of synaptic transmission is mediated by, at the least, a postsynaptic mechanism [Fig. 6(A)]. Further analysis of the time course of venom effect on sensory and agonist evoked PSPs, revealed that the disappearance and recovery of both kinds of PSPs follow the same time course [Fig. 6(B)]. Hence, there is no presynaptic effect that persists after the postsynaptic one for in such a case, the agonist-evoked response would have recovered sooner than the sensory evoked response. To conclude, the venom appears to interrupt synaptic transmission between cercal afferents and GIs by blocking the cholinergic receptors on the postsynaptic interneurons.

### Behavioral Effect of Thoracic Injection of a Cholinergic Antagonist

If the central block of cholinergic transmission can account for the flaccid paralysis of the front legs by the thoracic sting, we should be able to reproduce this paralysis by injecting a cholinergic antagonist in the thorax. To test this, we injected d-turbocurarine (TC) into the first thoracic ganglion (T1) in intact cockroaches. The concentration of TC ( $10^{-6}$  M) that we used was adjusted to the minimum concentration sufficient to induce a block of CCh evoked PSPs in a GI (data not shown) and is comparable to the concentration used by Blagburn and Sattelle (1987) to block synaptic transmission in the cockroach A6 ganglion. After recovering from the injection in T1, the injected cockroaches assumed a "head-down" posture very similar to that observed during the first few minutes following a sting [Fig. 1(C)]. A few minutes later, the injected cockroaches were able to walk using the middle and back legs while exhibiting a flaccid paralysis of the front legs, very similar to the paralysis caused by the thoracic sting of the wasp ( $n = 10$ ).

## DISCUSSION

### Site of the Thoracic Sting

Using radiolabeled venom, we have unequivocally shown that *Ampulex compressa* first stings its cockroach prey directly into the first thoracic ganglion. In a similar system, Gnatzy and Otto (1996) have provided indirect evidence that the wasp *Liris nigra*

stings its cricket prey in the thoracic and subesophageal ganglia (but not the brain ganglion). However, in their study, visual observation of the penetration of the sting into the nervous system was obtained under restrained conditions with a tethered wasp forced to sting a tethered prey.

Most solitary Sphecid wasps prey on large Orthopteroids equipped with various defense mechanisms (such as kicks, leaps, and bites). A common strategy applied by these wasps is a series of stings in the location of different thoracic ganglia accompanied by a sting to the neck of the prey. In all known cases, the first sting is directed at ganglia involved in locomotion and defense, thereby disarming the prey by inducing 2–60 min of complete paralysis (Steiner, 1986). For example, *Liris nigra* that hunts crickets first stings the third thoracic ganglion that controls the jumping legs (Steiner, 1986; Ferber et al., 1999), while *Tachysphex costi* and *Stizus ruficornis*, both preying on mantis, begin the stinging sequence with a sting to the first thoracic ganglion rendering the front, raptorial legs dangling paralyzed. Thus, we suggest that *Ampulex compressa* stings the cockroach directly into the first thoracic ganglion to flaccidly paralyze the front legs, thereby facilitating the more difficult and precise sting into the head. It is likely that other parasitoid wasps, including *L. nigra*, follow the same strategy of "drug delivery," injecting venom directly into the central nervous system of the prey.

### Ampulex Compressa's Venom Affects Cholinergic Synaptic Transmission

We have described the main initial effect of the venom by milking and injecting *Ampulex compressa* venom into specific ganglia in the cockroach while recording neuronal responses evoked electrically or pharmacologically. When injected into a thoracic ganglion, the venom blocked the evoked and spontaneous activity of motoneurons. The slow coxal depressor motoneuron (Ds) normally exhibits a tonic activity to maintain muscle tension required for leg posture. This tonic activity, which could be generated by synaptic input or endogenous properties, was abolished by the injection of venom and recovered within a few minutes. The venom also abolished the evoked response of both slow (Ds) and fast (Df) motoneurons for a few minutes. Because specific leg motoneurons, such as Df, are known to receive cholinergic innervation (Tornøe et al., 1995), venom could block cholinergic synaptic drive to Df and Ds as well as to other motoneurons. To explore this possibility, we used the sensory to giant interneuron synapse in the last abdominal ganglion (A6) of the cockroach to further

characterize the mechanism of action of the venom. This synapse has been extensively used as a pharmacological model for central cholinergic transmission (Callec and Sattelle, 1973; Piek et al., 1989; Hue and Callec, 1990). Carbachol (CCh), Nicotine, and Acetylcholine induce depolarizing postsynaptic potentials in the dendrites of GI3 in  $\text{Ca}^{2+}$  free conditions suggesting the existence of nicotinic receptors on GI3 dendrites (Blagburn and Sattelle, 1987). These depolarizing responses are blocked with nicotinic antagonists (such as Mecamylamine and d-Tubocurarine) but not affected by muscarinic antagonists (such as Atropine and Quinuclidinyl-benzilate) or a muscarinic agonist such as Oxotremorine (Blagburn and Sattelle, 1987). In the A6 preparation, the venom blocked cholinergic synaptic transmission and postsynaptic responses to a direct application of a nicotinic agonist for 2 to 3 min without blocking the propagation of action potentials. The time course of this synaptic block corresponds to the naturally occurring flaccid paralysis caused by the thoracic sting.

In insects, there are three types of receptors involved in cholinergic synaptic transmission: nicotinic, muscarinic, and mixed receptors (Hue and Callec, 1990). In *Manduca sexta*, presynaptic muscarinic receptors reduce the release of ACh from mechanosensory neurons (Trimmer and Weeks, 1989) or increase the excitability of postsynaptic motoneurons (Trimmer and Weeks, 1993) and in cockroaches, the activation of muscarinic receptors can downregulate nicotinic responses in Df (David and Pitman 1996). In contrast, in the sensory to GI system of the cockroach, both the pre- and postsynaptic neurons depolarize with the application of nicotinic agonists while muscarinic agonists and antagonists have no effect on presynaptic terminals or postsynaptic giant interneurons (Blagburn and Sattelle, 1987). Thus, the block of Carbachol evoked potentials that we have shown in the sensory to GI synapse strongly indicates a postsynaptic block of nicotinic receptors rather than a block of muscarinic receptors. Because the thoracic central synapses may also express muscarinic and mixed type receptors, we cannot rule out the possibility that the venom blocks muscarinic or mixed receptors on thoracic neurons. However, by reproducing the effect of the thoracic sting with an injection of a nicotinic blocker, we have demonstrated that such a nicotinic block is sufficient to explain the flaccid paralysis induced by the thoracic sting.

Finally, it is possible that some of the postsynaptic effects described in our study are due to activation of inhibitory synapses or other effects specific to the postsynaptic neuron. This would be consistent with the observed decrease in amplitude of the action po-

tentials recorded in the GI soma after venom injection. Furthermore, we cannot exclude presynaptic effects of the venom that are masked by the postsynaptic block of transmission. If such effects are mediated by different components of the venom, it should be possible to isolate them by direct application of individual venom components. We are currently fractionating these components and will examine the activity of each venom fraction on neuronal excitability and synaptic transmission.

## Wasp Venom Effects

Although the biochemical composition of venoms is known for only a few species of wasps (Piek and Spanjer, 1986; Piek, 1990), the venoms of solitary wasps vary greatly in composition and physiologic effects. Such effects of venoms vary from complete to incomplete and from permanent to a transient paralysis. Behavioral modulation of the envenomed prey occurs rarely as in the case of *Ampulex compressa* (Williams, 1942). Solitary wasps use a large repertoire of neurotoxins to induce paralysis in their prey through a block of central and peripheral synaptic transmission or through affecting voltage dependent conductances in nerve cells and muscle fibers. For instance, the crude venom of *Liris nigra* interrupts action potential propagation by blocking voltage dependent sodium currents in the cricket (Ferber et al., 2001). The insect glutamatergic neuromuscular synapse is presynaptically blocked by two *Microbracon* toxins (Piek et al., 1982) and postsynaptically blocked by Philanthotoxins (Eldefrawi et al., 1988). In the insect central nervous system, nicotinic synaptic transmission is blocked presynaptically by Kinins, which are small peptides found, for example, in the venom of *Megascolia flavifrons* (Piek et al., 1987) and postsynaptically by Philanthotoxins, which are polyamines found in the venom of *Philantus triangulum* (Piek and Hue, 1989). Thus, the transient paralysis described in this study seems to be similar in mechanism to that of Philanthotoxins. Indeed, when a nicotinic receptor blocker (d-tubocurarine) was injected into the first thoracic ganglion of cockroaches, their front legs were flaccidly paralyzed, and they assumed a posture similar to that of cockroaches stung by *Ampulex compressa*. However, there is no evidence for the existence of polyamines in *Ampulex's* venom. Likewise, biochemical analysis of the venom of *Ampulex compressa* and *Philantus triangulum* fails to reveal the presence of Kinins (Piek, 1990).

Venom compounds are useful tools to reveal physiologic, cellular, and molecular mechanisms underlying nerve cell physiology. Unlike the venom of most

other predators, *A. compressa*'s venom has no effect on the cockroach muscular system. In this study, we have uncovered a rather unique mode of delivery of the venom into a locomotory ganglion of its prey. This focal injection causes a local and specific modulation of motor control through a modulation of central synapses. The molecular components of the predator at 02'  $\Delta$  venom and their corresponding molecular targets in the prey, which are involved in this transient central paralysis, remain to be identified.

We would like to thank Lior Rosenberg for help with the radioactivity labeling experiments and Miriam Brodt for helpful comments on this manuscript. For their generous gifts of wasps we are very grateful to Mr. Wiederholz of the Aquazoo of Dusseldorf, P. Pearce-Kelly of The London Zoo, and K. Velman and E. Bruins of the Artis Zoo in Amsterdam. We would also like to thank the Kreitman fellowship and the Kreitman family for their support of Gal Haspel during his graduate studies. These experiments comply with the "Principles of animal care," publication No. 86-23 (revised 1985) of the National Institute of Health and also with the current laws of the State of Israel.

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