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ORIGINAL PAPER

A. Weisel-Eichler · F. Libersat

Are monoaminergic systems involved in the lethargy induced by a parasitoid wasp in the cockroach prey?

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Abstract The venom of the parasitoid wasp *Ampulex compressa* induces long-lasting hypokinesia in the cockroach prey. Previous work indicates that the venom acts in the subesophageal ganglion to indirectly affect modulation of thoracic circuits for locomotion. However, the target of the venom in the subesophageal ganglion, and the mechanism by which the venom achieves its effects are as yet unknown. While the stung cockroaches appear generally lethargic, not all behaviors were affected, indicating that the venom targets specific motor systems and not behavior in general. Stung cockroaches were observed "freezing" in abnormal positions. Reserpine, which depletes monoamines, mimics the behavioral effects of the venom. We treated cockroaches with antagonists to dopamine and octopamine receptors, and found that the dopamine system is required for normal escape response. Dopamine injection induces prolonged grooming in normal cockroaches, but not in stung, suggesting that the venom is affecting dopamine receptors, or targets downstream of these receptors, in the subesophageal ganglion. This dopamine blocking effect fades slowly over the course of several weeks, similar to the time course of recovery from hypokinesia. The similarity in the time courses suggests that the mechanism underlying the hypokinesia may be the block of the dopamine receptors.

Keywords Venom · Wasp · Dopamine · Hypokinesia · Cockroach

Abbreviations *SEG* subesophageal ganglion

A. Weisel-Eichler (✉) · F. Libersat
Department of Life Sciences
and Zlotowski Center for Neuroscience,
Ben-Gurion University of the Negev,
Beer Sheva, Israel
E-mail: aviva@alum.mit.edu
Tel.: +972-8647-2112
Fax: +972-8647-9216

Introduction

The venoms of several species of parasitoid sphecid wasps have been shown to induce a lethargic state in their prey (Piek and Spanjer 1986). The prey are not paralyzed but show little initiation of spontaneous or provoked movement (Williams 1942; Piek and Spanjer 1986; Fouad et al. 1994). The mechanism by which the venom produces this change in responsiveness and activity level is not known. Understanding the mechanism of the venom action can give us insights into the normal control of initiation of behavior.

The parasitoid sphecid wasp *Ampulex compressa* stings the cockroach *Periplaneta americana* into the head, towards the subesophageal ganglion (SEG) (Fig. 1). The venom of *A. compressa* has three sequential behavioral effects on the cockroach. During the first 2–5 min following the sting into the head, the cockroach is immobile (Williams 1942; Piek et al. 1984). For the next 30–40 min the cockroach performs excessive grooming behavior (Weisel-Eichler et al. 1999). After this, and lasting for several weeks, the cockroach is lethargic (Williams 1942; Piek et al. 1984; Piek et al. 1989; Fouad et al. 1994). This paper focuses on the third, long-term effect of the venom. During this time, cockroaches stung by the wasp are unable to generate wind- or touch-evoked escape behavior (Fouad et al. 1994). In normal cockroaches, wind-evoked escape behavior is initiated when the cerci sense a puff of wind (Fig. 1). Sensory neurons in the cerci synapse monosynaptically onto giant interneurons in the last abdominal ganglion. The giant interneurons project to the locomotory centers in the three thoracic ganglia. There they activate interneurons, which, in turn, activate motoneurons involved in producing the escape leg movements (Camhi 1984; Ritzmann and Pollack 1986).

For *A. compressa*, it has been shown that the venom does not affect the motoneurons, neuro-muscular junction or muscle response (Fig. 1). Nor does the venom affect activation of the thoracic interneurons by primary

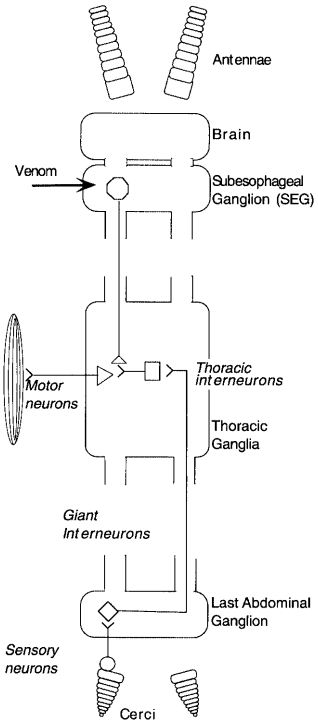

Fig. 1. Model of venom-induced depression of the escape circuit by the wasp neurotoxin. Sensory neurons in the cerci are stimulated by wind puffs. These sensory neurons excite giant interneurons which activate thoracic interneurons involved in producing escape behavior. These, in turn, activate thoracic motoneurons controlling the leg muscles. The venom may target specific neurons in the subesophageal ganglion that, directly or indirectly, regulate the excitability of the thoracic portion of the escape circuitry by modulating the synapses between thoracic interneurons and specific motoneurons. (Modified from Libersat et al. 1999)

sensory interneurons – giant interneurons and others (Fouad et al. 1994, 1996; Libersat et al. 1999). Therefore, the thoracic site that is indirectly modulated by the venom injected into the head, appears to be the synaptic connections between thoracic interneurons and thoracic motoneurons for locomotion (Libersat et al. 1999). Since the venom is injected by the wasp into the SEG, it is likely that the venom directly affects SEG neurons that modulate synapses in the thorax.

The SEG has been found to be involved in selection, organization, initiation and maintenance of various behaviors in insects, such as walking (Kien 1983; Altman and Kien 1987), stridulation (Lins and Elsner 1995), flight (Ramirez 1988), grooming (Weisel-Eichler et al. 1999), and ventilation (Otto and Janiszewski 1989). Results suggest that the SEG functions to control the excitability of motor networks in the thorax (Altman and Kien 1987; Johnston et al. 1999). In the locust, the SEG drives efferent octopaminergic neurons in the thorax that provide neuromodulatory input to the leg muscles (Duch et al. 1999).

In order to help reveal the target of the venom in the SEG, with the ultimate goal of identifying the impaired system, we sought to further characterize the deficits in the behavioral repertoire of stung cockroaches. In particular, we wished to determine if the venom affects all behaviors or only specific behaviors. To gain insight into the mechanism by which the venom produces the long-term behavioral effects, we treated animals with reserpine as a pharmacological model. Reserpine was chosen because this plant alkaloid produces long-term lethargy, but not paralysis, in many animals, including cockroaches (Sloley and Owen 1982), an effect resembling that of the venom. At the cellular level, reserpine acts by depleting monoamines for several weeks (Frontali 1968; Sloley and Owen 1982), which is similar to the duration of the lethargic state in stung cockroaches (Fouad et al. 1994). In insects, the monoamines depleted are dopamine, serotonin, and octopamine (Robertson 1976; Sloley and Owen 1982). To determine which of these monoamines might be involved in producing the venom effects, we also used specific antagonists as pharmacological models. Following this, we focused on the dopaminergic system as a possible target of the venom. In this paper we show that the venom of *A. compressa* affects dopamine receptors in the SEG of the cockroach prey.

Materials and methods

Animals

All experiments were performed on adult male cockroaches *P. americana*. Cockroaches and wasps were raised as described in Weisel-Eichler et al. (1999). We obtained stung cockroaches by placing a cockroach with a female wasp and allowing the wasp to sting the cockroach with the species-specific stinging sequence, which includes a sting into the head directed at the subesophageal ganglion (see Fouad et al. 1994 for description). Cockroaches that were tested one or more days after being stung or injected with reserpine were kept in individual, small plastic containers with food and water. Testing was done 2–9 h after the beginning of the light cycle, at room temperature.

Behavior

To assess spontaneous behavior, we observed cockroaches in a round arena, which was 60 cm in diameter and covered with Plexiglas. We placed a small piece of food and a small dish of water near the center. The walls were covered with Vaseline to prevent the cockroaches from climbing onto the cover. We introduced the cockroach into the center of the arena without touching it. The arena was placed so that no movements outside the arena would be visible to the animals. Illumination was provided by fluorescent lights on the ceiling. We observed for 30 min and recorded the behavior and location of the animal each minute on the minute.

We observed the activity level of cockroaches in the arena, which had markings on the floor dividing the area into four quadrants. After placing the cockroach in the arena, we waited 5 min for it to acclimatize, and then counted the number of times the animal crossed into a different quadrant during the next 10 min.

The escape response was tested as follows: Cockroaches were placed in the arena and given 5 min to acclimatize. Following this we placed a plastic cup over the cockroach and gently moved the animal into the center of the arena. When the cockroach was calm the cup was removed, and the end of the wings was touched very lightly with the bristles of a small paint brush. If the cockroach ran to the wall of the arena this was scored as a positive response; if the

cockroach made only a few steps or did not respond, this was scored as a negative response. Intermediate responses were rare and were discarded (3 animals out of 67).

We observed grooming behavior of cockroaches in an opaque plastic box (29 cm×18 cm×13 cm), with a clear plastic cover. The floor of the box was covered with small pebbles. Using a stopwatch, we measured the amount of time spent grooming during the 30 min period immediately following treatment. Grooming behavior was defined as described in Weisel-Eichler et al. (1999). Animals had never been in the testing box previously, and thus it was a novel environment in all cases. The "stressed" group of cockroaches was stressed by handling for 2 min by an experimenter wearing clean gloves. Cockroaches that were sprayed with irritant received two overall sprays of a solution of 5% acetic acid, an irritant that elicits prolonged grooming behavior in insects, as described in Hogan-Warburg et al. (1995) and Weisel-Eichler et al. (1999).

Righting behavior was filmed from above with a black and white Sony CCD video camera at 25 frames s^{-1}. We placed a cockroach on its back using forceps to hold the pronotum in such a way as to prevent the cockroach from grabbing on to the forceps with its legs. The surface was course sandpaper. Each animal was tested four times. Video films were analyzed off-line using a JVC video cassette recorder (SR S388E) with frame grabber. We counted the frames from the time the forceps released the cockroach until the cockroach stood upright.

Flight behavior was tested by holding the cockroach suspended in a wind tunnel. The cockroach was held by the pronotum with forceps. The wind tunnel was 14 cm×14 cm (height×width), and the wind velocity measured at the opening, with an anemometer, was 2 m s^{-1} (800 vtp Datametrics, Watertown, Mass., USA). When suspended in the wind tunnel, cockroaches responded in one of three ways: (1) the animal hung from the forceps without opening the wings; (2) the animal opened the winds and flapped them a few times; or (3) the animal opened the wings and sustained flight for at least 3 s. The first two cases were scored as a negative response. The third case was scored as positive.

Drugs

All pharmacological solutions were prepared fresh on the day of the experiment. For drugs administered via the hemolymph, 10 µl (18 µl for mianserin) of solution was injected between the 4th and 5th sternites into the abdominal hemocoel with a Hamilton syringe. Dopamine (Sigma), flupentixol (Research Biochemical International), and mianserin (Sigma) were prepared in cockroach saline (Blagburn and Sattelle 1987). Dopamine was prepared at 10^{-5} mol l^{-1}; flupentixol at 2×10^{-2} mol l^{-1} (200 nmol was injected); and mianserin at 10^{-2} mol l^{-1} (200 nmol was injected). Reserpine (Sigma) was prepared in safflower oil at 5×10^{-3} mol l^{-1} (2×10^{-3} mol l^{-1} for SEG). SKF-82958 (Research Biochemical International) for injection into the SEG was dissolved at 10^{-8} mol l^{-1} in a cockroach saline suitable for injection into the CNS, of the following composition (in mmol l^{-1}): NaCl 214; KCl 3.1; $CaCl_2$ 9; sucrose 50; Hepes buffer 5; pH 7.2, based on Wafford and Sattelle (1986). To inject into the SEG, we immobilized cockroaches by covering them with modeling clay. We made a slit in the ventral cuticle between the neck and the head and injected 10 nl of solution into the posterior medial area of the SEG using a nano-volumetric injector (Medical Systems, N.Y.). Injection of SKF 82958 into this area of the SEG has been shown previously to induce grooming (Weisel-Eichler et al. 1999). Placement of the pipette was controlled visually using a stereo-microscope (Olympus). Cockroaches that did not begin walking immediately after surgery were considered to have not recovered sufficiently from surgery and were not included in the sample.

Data analysis

All values are given as mean ± standard deviation (SD), unless otherwise indicated. Statistical tests were done using GB Stat version 5.0.6, except for ANOVA which was done with Systat version 9, and Fisher's exact test which was calculated using formulas in Sokal and Rohlf (1995). We used a nested ANOVA to analyze the data from the righting experiment because each animal was tested four times.

Results

Spontaneous and provoked behaviors

We examined various spontaneous behaviors in an arena to see which behaviors are affected by the venom. We also examined three provoked behaviors: righting behavior, flight in a wind tunnel, and grooming performed in response to stress or an irritant. Stung cockroaches were tested 1 day after the sting.

Stung cockroaches were observed in an arena, and their behavior was recorded once per minute. Stung cockroaches were observed walking or running significantly less often than controls (Fig. 2A). There was no significant difference between stung cockroaches and controls in the number of times grooming or wall seeking behavior were observed (Fig. 2B, C). At the beginning of each experiment, the cockroach was released in the center of the arena. The results indicate that even stung cockroaches generally moved from the center to spend most of their time next to the wall, the preferred location for normal cockroaches. Eating and drinking were observed so rarely in both groups that this behavior was not included in the analysis. However, stung cockroaches do eat and drink (A. Weisel-Eichler and F. Libersat, unpublished observations).

Three provoked behaviors were examined: grooming, righting and flight. There was no significant difference between stung and controls in the amount of grooming behavior performed during the 30 min following stress (Fig. 3A), or spray with an irritant (Fig. 3B). There was also no significant difference between stung and controls in the amount of time required to turn over and stand on the legs after being placed on the back (Fig. 3C). All of the stung and all of the control cockroaches flew when suspended in a wind tunnel (Fig. 3D).

While observing stung cockroaches in this and other behavioral experiments, we often observed them adopting a "frozen" position, which was quite striking in its abnormality. There were two general types of freezing noticed: (1) the animal froze in the middle of a grooming movement with the mouth on a leg, an antenna, or the thorax; and (2) the animal froze with the body axis tilted up, and/or with one prothoracic leg in the air. Normal animals, when motionless, have their body axis parallel, or nearly parallel, to the substrate (floor or wall), and all six legs on the substrate. We never observed this freezing behavior in over 30 h observing 60 control cockroaches. In the arena experiment, 4 out of 11 stung cockroaches, observed 2–24 h after being stung, were observed in a "frozen" position. Several episodes of freezing were observed for each of the four animals, each episode lasting between 1 min and 8 min.

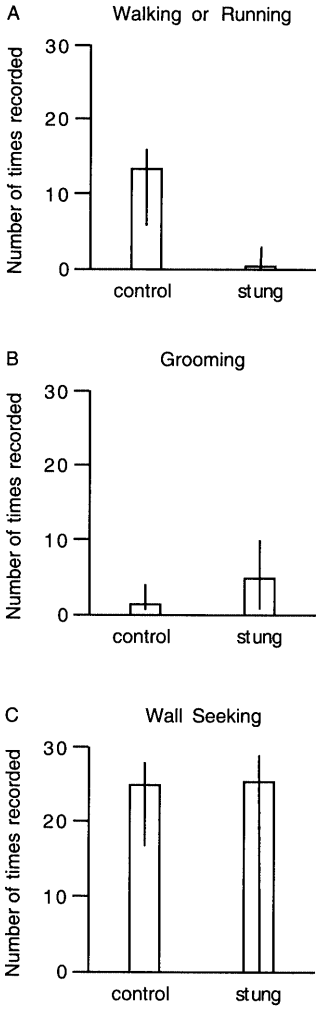

Fig. 2A,B. Not all spontaneous behaviors are affected by the venom. *Y*-axis indicates number of times each of the indicated behaviors was recorded during a 30-min observation period (sampled once per minute). Data shown are medians and 75% of the range. **A** *Walking or running* is significantly reduced in stung cockroaches ($P < 0.01$; Mann-Whitney *U*-test; $n = 8$ each). **B** *Grooming* is not significantly affected ($P = 0.1$; Mann-Whitney *U*-test; $n = 8$ each). **C** *Wall seeking* is not significantly affected ($P = 0.8$; Mann-Whitney *U*-test; $n = 8$ each)

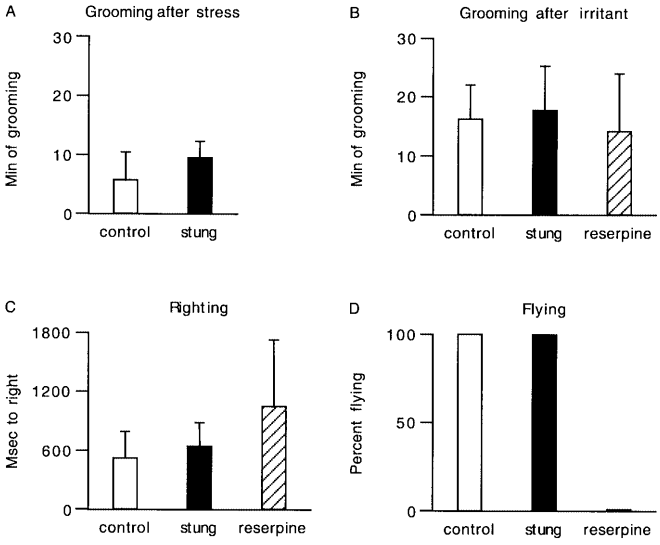

Fig. 3A–D. Effect of venom and of reserpine on cockroach behaviors. **A** *Y*-axis indicates minutes spent grooming during the 30 min following stress. There is no significant difference between stung and control ($P = 0.1$; *t*-test; $n = 8$ each). **B** *Y*-axis indicates minutes spent grooming during the 30 min following spray with an irritant. There are no significant differences among the three groups ($P > 0.4$; *t*-test; $n = 8$ each). **C** *Y*-axis indicates time required for a cockroach to turn over after being placed on its back. There is no significant difference between stung and controls ($P = 0.3$). Reserpine-treated take significantly more time than controls ($P < 0.001$), or than stung ($P < 0.01$) (nested ANOVA; $n = 10$ each). **D** *Y*-axis indicates percent of animals that flew when placed in a wind tunnel. There was no difference between stung and controls. Reserpine-treated flew significantly less than controls or stung ($P < 0.01$; Fisher's exact test; $n = 8$ each). In **A**, **B**, and **C** data are means + SD

Reserpine as a pharmacological model for the venom

We sought to explore the possibility that the venom is acting via a mechanism similar to that of the drug reserpine, which induces lethargy in insects by depleting the monoamines octopamine, dopamine and serotonin (Robertson 1976; Sloley and Owen 1982). Therefore, we compared the behavioral effects of reserpine to those of the venom. We injected cockroaches with reserpine into the hemolymph and observed them the next day. Reserpine-injected animals showed little spontaneous or provoked locomotion; they walked, but did not run, similar to stung cockroaches. In addition, reserpinized animals showed the same posture as stung animals – body low and parallel to the floor, head down. Quantitative analysis of behavior showed a significant reduction in spontaneous activity, as compared to vehicle injected animals (Fig. 4A). The activity level of reserpinized cockroaches was similar to that of stung animals (Fig. 4A).

Animals injected with reserpine directly into the SEG did not show an escape response when touched lightly, while vehicle-injected animals escaped normally (Fig. 4B). This was similar to stung animals where none of the stung cockroaches escaped when touched, while all control cockroaches escaped in all trials (Fig. 4B; as previously described by Fouad et al. 1994). Animals injected with reserpine into the hemolymph also did not show an escape response, while animals injected with vehicle had normal escape responses (Fisher's exact test; $P < 0.01$; $n = 8$ each). The escape response in reserpine-treated animals recovered after 4 weeks (not shown), similar to the time-course of recovery from venom in stung animals (Fouad et al. 1994).

As described above, grooming behavior in response to an irritant is not compromised in stung animals, and the same was true for animals injected with reserpine into the hemolymph (Fig. 3B). However, in contrast to stung animals whose ability to right is not impaired, reserpine-treated animals, while able to perform righting behavior, were significantly slower (Fig. 3C). In addition, while all stung cockroaches flew when placed in a

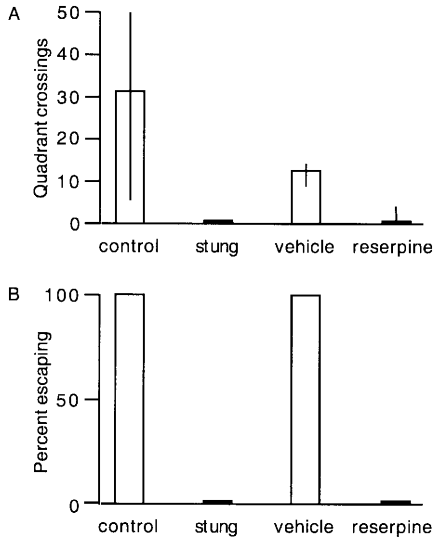

Fig. 4A,B. Reserpine reduces activity level and compromises escape response. **A** *Y*-axis indicates number of times animal crossed quadrants in 10 min. One day after being stung, cockroaches have significantly lower activity level than controls ($P < 0.001$; Mann-Whitney *U*-test; stung: $n = 5$, controls: $n = 8$). One day after injection of reserpine into the hemolymph, the activity level of animals was significantly lower than vehicle-injected ($P < 0.01$; Mann-Whitney *U*-test; $n = 5$ each). Data shown are medians and range. **B** *Y*-axis indicates percentage of cockroaches responding with an escape response to a light touch. One day after being stung, cockroaches escape significantly less often than controls ($P < 0.01$; Fisher's exact test; $n = 8$ each). One day after injecting reserpine into the subesophageal ganglion (SEG), cockroaches escape significantly less often than vehicle injected ($P < 0.01$; Fisher's exact test; $n = 6$ each). For both **A** and **B** *vehicle* is the oil in which reserpine was dissolved

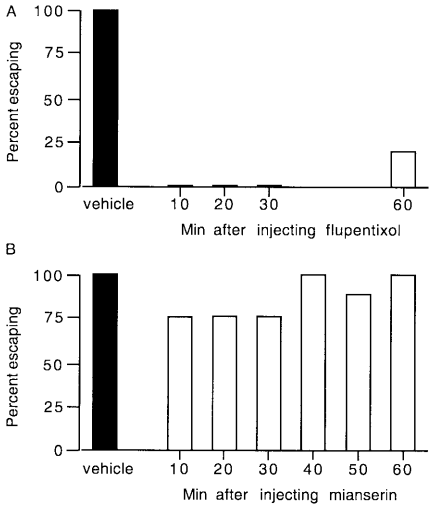

Fig. 5A,B. Flupentixol affects escape response; mianserin does not. *Y*-axis indicates percentage of cockroaches responding with an escape response to a light touch. **A** After injecting flupentixol into the hemolymph there was no escape response beginning 10 min after injection ($P < 0.01$; Fisher's exact test; $n = 10$ each). **B** After injecting mianserin into the hemolymph there was no significant effect on escape response during any of the tests 10–60 min following injection (Fisher's exact test; $n = 8$ each). In both **A** and **B** the vehicle used was saline

wind tunnel, none of the reserpine-treated cockroaches flew (Fig. 3D).

The results of the experiments with reserpine-treated cockroaches showed that reserpine mimics the behavioral effects of *A. compressa* venom, but has some additional effects, as well. Reserpine depletes the monoamines dopamine, octopamine and serotonin. To determine which of the monoamines might be affected by the venom, we further examined the escape response since it is dramatically affected by both reserpine and the venom. We treated animals with antagonists for monoamines. The dopamine D1/D2 receptor antagonist flupentixol, injected into the hemolymph, suppressed the escape response (Fig. 5A). However, mianserin, an octopamine receptor antagonist, did not affect the escape response (Fig. 5B). This indicates that the escape response requires normal functioning of the dopamine receptors in the cockroach.

Dopamine-induced grooming

In normal cockroaches, injection of dopamine induces prolonged grooming (Weisel-Eichler et al. 1999). We used this response to test the functioning of the dopamine receptors in stung cockroaches. Dopamine injected into the hemolymph of cockroaches that had been stung by *A. compressa* the day before and were in the lethargic state did not induce the prolonged grooming which is seen in normal cockroaches (Fig. 6A). Similarly, the dopamine D1 agonist SKF 82958, injected directly into the SEG, induced prolonged grooming in normal animals but not in stung (Fig. 6B). Reserpine, injected into the hemolymph, causes massive release of dopamine and other monoamines from nerve terminals (Oates 1996), and induced prolonged grooming in normal cockroaches, but not in stung (Fig. 6C). Injecting dopamine, SKF 82958 or reserpine into stung cockroaches induced a level of grooming similar to that seen after injecting the corresponding vehicle into normal cockroaches (not shown). The venom of *A. compressa*, which contains dopamine or a dopamine-like substance normally induces very intensive grooming for about 40 min after the sting, before the lethargic effect begins (Weisel-Eichler et al. 1999). However, if *A. compressa* stings a cockroach that has been stung previously, and is already in the lethargic state induced by the first sting, then this second sting does not induce prolonged grooming (Fig. 6D). This lack of responsiveness of stung cockroaches to all of the above treatments was not due to their general lethargy, since, as shown above (Fig. 3A, B), stung cockroaches groom as much as normal insects in response to stress or spray with an irritant. Therefore, it appears that dopamine receptors on cells involved in grooming are compromised in the SEG of stung cockroaches.

Other than the dopamine or dopamine-like substance in the venom, no other venom component has, as yet, been identified. For the purposes of this paper, we will refer to the venom component that blocks

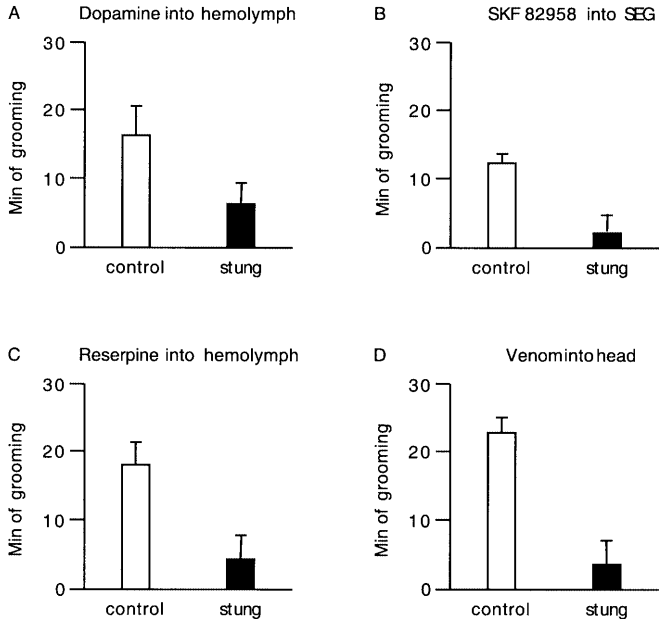

Fig. 6A–D. Pharmacologically induced prolonged grooming is not seen in stung cockroaches. *Y*-axis indicates the time spent grooming during the 30 min following injection. **A** Injection of dopamine into the hemolymph of cockroaches stung the day before induces significantly less grooming than injection of dopamine into normal cockroaches ($P < 0.001$; *t*-test; $n = 8$ each). **B** Injection of the dopamine D1 agonist SKF 82958 into the SEG of cockroaches stung the day before induces significantly less grooming than injection of SKF 82958 into normal cockroaches ($P < 0.001$; *t*-test; $n = 4$ control, $n = 6$ stung). **C** Injection of reserpine into the hemolymph of cockroaches stung the day before induces significantly less grooming than injection of reserpine into normal cockroaches ($P < 0.001$; *t*-test; $n = 8$ each). **D** Sting by the wasp *A. compressa* in cockroaches that had been stung previously the day before induces significantly less grooming than a sting in normal cockroaches ($P < 0.0001$; *t*-test; $n = 8$ each). In all graphs data are means + SD

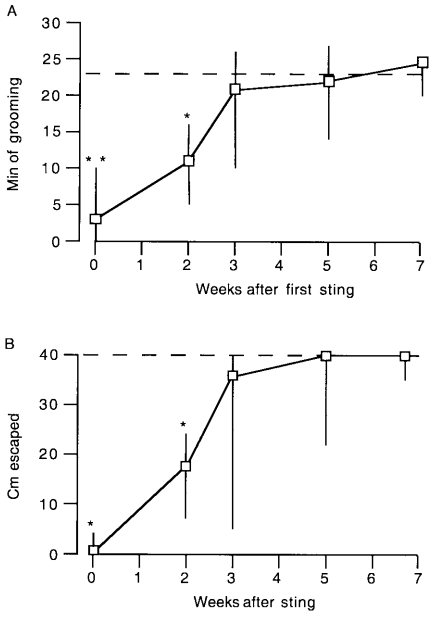

Fig. 7A,B. Time-course of recovery from venom blocking of venom-induced grooming resembles recovery from lack of escape response. **A** *Y*-axis indicates amount of grooming induced by a *second* sting. *X*-axis indicates time between first sting and second sting. *Dotted line* indicates median minutes of grooming induced by a *first* sting ($N = 8$). **B** *Y*-axis indicates distance cockroach ran after being touched lightly ($n = 6$) (adapted from Fouad et al. 1994). The maximum possible distance in this experiment was 40 cm. *Dotted line* indicates that 100% of control cockroaches ran the maximum distance. **A** and **B** Data are median and range. Time "0" is 1 day after first sting. Groups at 0 weeks and at 2 weeks were significantly different from controls $^{**}P < 0.001$; $^{*}P < 0.01$ (Mann-Whitney *U*-test)

venom-induced grooming in previously stung cockroaches as "dopamine-blocking" component. We will refer to the component that induces lethargy and hypokinesia as "hypokinesia-inducing" component. It should be understood that each of the two components defined here may actually consist of more than one substance, and, also, that these two components may be partially or fully equivalent to each other.

By subjecting cockroaches to a second sting at various times after the first sting, we found that the venom blocking of dopamine-induced grooming, the effect of the dopamine-blocking component, faded slowly and disappeared after about 3–5 weeks (Fig. 7A). In other words, a second sting, administered 5 weeks after a first sting, elicited a level of excessive grooming similar to that seen after a first sting. There was a striking similarity between this time-course and the time-course for recovery of the escape behavior described by Fouad et al. (1994; see Fig. 7B), which reflects the time-course of the hypokinesia-inducing component of the venom. The similarity in the time-courses suggests a common mechanism for the venom blocking of dopamine-induced grooming and the venom-induced hypokinesia.

Discussion

In this study we show that the venom of *A. compressa* targets specific motor systems and not behavior in general. Also, the venom has a blocking effect on dopamine receptors in the subesophageal ganglion of stung cockroaches. This prevents dopamine-induced grooming and may also underlie the long-term hypokinesia seen in stung cockroaches.

Venom effects on behavior

Cockroaches stung by the parasitoid wasp *A. compressa* show very little spontaneous or provoked activity. The method of locomotion was also different. Normal cockroaches generally made short, quick, darting, running movements, broken up by periods of immobility. Stung cockroaches, on the other hand, when they moved, walked in a slow, steady manner (A. Weisel-Eichler and F. Libersat, unpublished observations). However, while stung cockroaches perform very little spontaneous locomotion, in most cases they moved from their starting position in the center of the arena to a position next to the wall of the arena (Fig. 2). So although the escape reflex is

depressed (Fouad et al. 1994), the thigmotaxis reflex appears to be active. In addition, while stung cockroaches were less frequently observed in locomotion behaviors involving the legs – walking, running (Fig. 2) – we saw no difference between stung and control cockroaches in spontaneous or provoked grooming, righting behavior, or ability to fly in a wind tunnel (Figs. 2, 3).

These results indicate that the venom affects motor systems selectively, rather than affecting all motor systems and behaviors. This specificity may be achieved by targeting a certain neuromodulatory system that controls a specific subset of behaviors. Such specificity of neuromodulatory systems has often been observed in invertebrates, where specific neuromodulators, particularly the monoamines, have been found to release well-defined behaviors. For example, serotonin releases swimming and biting in the leech (Lent et al. 1989); aggressive and submissive postures in the lobster are induced by serotonin and octopamine respectively (Kravitz 1988); dopamine induces grooming in the cockroach (Weisel-Eichler et al. 1999); and octopamine releases flight in the moth and locust (Sombati and Hoyle 1984; Stevenson and Kutsch 1987).

Pharmacological models

Reserpine causes massive release and subsequent depletion of monoamines (reviewed in Oates 1996). Thus, depletion of the monoamines mimics the lack of spontaneous activity, the posture, and the lack of an escape response seen in stung animals (Fig. 4). Reserpine affects escape response when injected directly into the SEG, showing that depletion of monoamines in the SEG alone is sufficient to suppress escape response. Reserpine-treated animals resembled stung animals in their ability to perform intensive grooming after being sprayed with an irritant. Reserpine-treated animals partly resembled stung animals in that they showed a righting response, although the reserpine-treated animals were slower to complete righting (Fig. 3). These similarities between reserpine and venom suggested to us that the venom was acting by affecting one or more monoaminergic systems in the cockroaches. In contrast to stung animals, reserpine-treated animals had the additional impairment of inability to fly, which was not seen in the stung animals (Fig. 3). This may be because reserpine injected into the hemolymph affects monoamines in the peripheral nervous system, as well as the central nervous system, while the venom probably only affects the central nervous system (L. Rosenberg et al., unpublished observations). Another explanation is that the venom may be affecting fewer monoaminergic systems than reserpine.

To determine which of the depleted monoamines might be responsible for the lack of escape response, we treated animals with specific antagonists. Animals treated with the dopamine D1/D2 receptor antagonist flupentixol, an effective antagonist of dopamine receptors in cockroach brain (Orr et al. 1987), did not show an escape response. However, at least 75% of animals treated with mianserin, an effective antagonist of octopamine receptors in cockroach brain (Orr et al. 1987), showed normal escape response (Fig. 5). Flupentixol is a strong antagonist of dopamine receptors in cockroach brain and a weak antagonist of octopamine receptors (Orr et al. 1987). However, it is unlikely that flupentixol is blocking escape by acting on octopamine receptors since mianserin had little effect on escape. On the contrary, it is likely that the small effect of mianserin was due to its being a weak antagonist of dopamine receptors (Orr et al. 1987).

Since a dopamine receptor antagonist mimics the lack of escape response seen in stung animals, while an octopamine receptor antagonist does not, this suggests that a dopaminergic system is the target of the venom. In addition, these results suggest that it is unlikely that an octopaminergic system is the target of the venom. Due to the lack of specific antagonists for receptors of other monoamines in the cockroach CNS, including serotonin, we were not able to test other monoaminergic systems. Therefore, we have not eliminated the possibility that the venom affects other neuromodulatory systems, or subtypes of systems, besides the dopaminergic system.

Dopamine-blocking effect of the venom

Stress-induced and irritant-induced grooming behavior in stung cockroaches is comparable to that seen in unstung cockroaches (Fig. 3). Therefore, the lack of an extended grooming response after various dopamine related treatments that induce grooming in normal cockroaches (Fig. 6) suggests that the venom disables dopamine receptors. It appears that the targeted system is in the SEG because two of the dopamine related treatments (SKF 82958 and venom) were specifically directed at the SEG.

The identity of this surmised venom component that acts on, or downstream of, dopamine receptors is not known; other than the dopamine-like substance found by Weisel-Eichler et al. (1999), no other active venom component has yet been identified. As mentioned above, we have called this component dopamine-blocking component. However, it is possible that the venom effects are actually produced by interactions among several components. Interactions of venom components in other venoms is known to occur. The typical cone snail venom has been found to contain about 100 neuropharmacologically active components, mostly small peptides (Olivera 1999). It has been shown that combinations of these venom peptides act synergistically to produce specific behavioral effects (Olivera 1997).

One possible mechanism by which the venom may be affecting dopamine receptors is by causing a reduction in the density of functioning dopamine receptors. Various other venoms have been shown to act by binding to receptors for specific neurotransmitters and neuromodulators (reviewed in Adams and Swanson 1994). It is

possible, however, that the venom does not act directly on dopamine receptors but, rather, on the intracellular signaling pathway triggered by dopamine binding to receptors. We include this possibility when we say that the venom targets "dopamine receptors". Therefore, another possible site of action of *A. compressa* venom is inside the cell, directly on the G-protein or on adenylate cyclase. Such a mechanism is seen in the actions of mastoporan, a venom component of vespid wasps. Mastoporan, a 14-amino acid peptide, is an amphiphilic cation which has been postulated to cross the plasma membrane in response to resting membrane potential, and interact directly with G-proteins on the cyptoplasmic side of the cell membrane (Higashijima et al. 1988). It has been found to stimulate G-protein activity, mimicking an agonist (Higashijima et al. 1988). An additional possibility is that the venom acts on cells involved in generating grooming behavior that are downstream of the cells bearing the dopamine receptors.

Hypokinesia

Is the venom-induced hypokinesia produced by the dopamine-blocking component of the venom? We suggest that this is the case for the following reason. The time course of the recovery from dopamine-blocking component is unusual in that a single dose of venom has an effect which lasts for several weeks. This unusual time-course is also seen in the recovery from hypokinesia (Fig. 7). This suggests that a common mechanism is responsible for both the effects – hypokinesia and block of dopamine-induced grooming. Thus, the hypokinesia may also be due to a dysfunction in, or downstream of, dopamine receptors; dopamine-blocking and hypokinesia-inducing components of the venom may be identical.

It has been shown that the SEG is involved in controlling the excitability of motor networks in the thorax (Altman and Kien 1987; Johnston et al. 1999). It is possible that the cells in the SEG that bear dopamine receptors and are affected by the venom are part of this system for regulating activity level in the thorax. Many venoms have evolved special properties and are highly subtype specific (Adams and Olivera 1994). It appears that the venom of *A. compressa* has evolved to specifically target a neuromodulatory system to produce hypokinesia in the prey.

Dopamine in insects

Dopamine, dopaminergic cells, and dopamine receptors have been demonstrated in the insect central nervous system (Evans 1980; Horner 1999; Notman and Downer 1987; Kokay and Mercer 1996); however, relative to the information available for mammals, very little is known of the function of dopamine in the central nervous system or of the nature of the dopaminergic receptors in insects. Unlike mammalian dopamine receptors, which have been categorized into the D1 group and the D2 group, there is, as yet, no clear way to categorize insect dopamine receptors.

There are approximately ten bilateral pairs of dopaminergic cells in the SEG of the cockroach, distributed in all three neuromeres. The largest pair, SN1, located in the ventral anterior area of the SEG, innervate the salivary glands. Anterior to SN1 is a cluster of smaller dopaminergic cells which may project to the supraesophageal ganglion; a posterior pair of dopaminergic cells may project posteriorly in the ventral nerve cord (Elia et al. 1994). In addition, several areas in the SEG have dense dopaminergic innervation. This innervation derives from cells in the supraesophageal ganglion, the prothoracic ganglion, and other, as yet, unidentified sources (F. Libersat, unpublished results).

In insects, in the head ganglia, dopamine modulates olfactory processing (Macmillan and Mercer 1987), is necessary for the motor patterns of the proboscis extension reflex (Menzel et al. 1999), is involved in learning (Neckameyer 1998a), is necessary for normal female receptivity (Neckameyer 1998b), and induces grooming (Weisel-Eichler et al. 1999). In the ventral nerve cord, dopamine elicits flight (Claassen and Kammer 1986), potentiates escape response (Casagrand and Ritzmann 1992; Goldstein and Camhi 1991), stimulates locomotion (Yellman et al. 1997), and has an excitatory effect on motor neurons (Pitman and Baker 1989).

In vertebrates dopamine has been found to be involved in voluntary locomotor control, sexual function, cardiovascular homeostasis, endocrine regulation, emotion and cognition (reviewed in Hantraye 1998). The effect of dopamine on locomotion is complex, depending on, among other factors, whether D1 or D2 receptors are activated, which specific agonists and combinations of agonists are used, dose, and the site of action of the agonists in the brain (reviewed in Jackson and Westlind-Danielsson 1994). Massive loss of dopamine neurons in the substantia nigra in humans results in Parkinson's disease. One of the main characteristics of Parkinson's disease is paucity of voluntary movements, or hypokinesia. In experimental animals this symptom can be reproduced by treatment with reserpine or with dopamine receptor antagonists (reviewed in Hornykiewicz 1975). Paucity of movements also characterizes cockroaches stung by *A. compressa*. Another symptom of Parkinson's disease is freezing during movements (Fahn 1995). Freezing was also seen in the stung cockroaches.

The venom of *A. compressa* appears to target the SEG. One of the known functions of the SEG in insects is initiation of behaviors (Kien 1983; Altman and Kien 1987; Lins and Elsner 1995; Ramirez 1988; Otto and Janiszewski 1989; Weisel-Eichler et al. 1999). This study suggests that in insects dopamine plays a role in the SEG in initiation of locomotion and escape.

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