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OCTOPAMINE MIMICS THE EFFECTS OF PARASITISM ON THE FOREGUT OF THE TOBACCO HORNWORM *MANDUCA SEXTA*

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Summary

The parasitic braconid wasp *Cotesia congregata* lays its eggs inside the body of the larval stage of its host, the moth *Manduca sexta*. The *Cotesia congregata* larvae develop within the hemocoel of their host until their third instar, when they emerge and spin cocoons and pupate on the outer surface of the caterpillar. From this time until their death approximately 2 weeks later, the *Manduca sexta* larvae show striking behavioral changes that include dramatic declines in spontaneous activity and in the time spent feeding.

Coincident with these behavioral changes, it is known that octopamine titers in the hemolymph of the host become elevated by approximately 6.5-fold. Octopamine is an important modulator of neural function and behavior in insects, so we examined hosts for neural correlates to the behavioral changes that occur at parasite emergence. We found that, in addition to the changes reported earlier, after parasite emergence (post-emergence), *Manduca sexta* larvae also showed marked deficits in their ability to ingest food because of a disruption in the function of the frontal ganglion that results in a significant slowing or the absence of peristaltic activity in the foregut. This effect could be produced in unparasitized fifth-instar larvae by application of blood from post-emergence parasitized larvae or of 10^{-6} mol l^{-1} D,L-octopamine (approximately the level in the hemolymph of post-emergence larvae). In contrast, blood from parasitized larvae before their parasites emerge or from unparasitized fifth-instar larvae typically had no effect on foregut activity. The effects of either post-emergence parasitized blood or 10^{-6} mol l^{-1} octopamine could be blocked by the octopamine antagonists phentolamine (at 10^{-5} mol l^{-1}) or mianserin (at 10^{-7} mol l^{-1}).

Key words: biogenic amine, octopamine, parasitism, stomatogastric, *Cotesia congregata*, ingestion, feeding, insect, tobacco hornworm, *Manduca sexta*.

Introduction

Parasites, evolving in close association with their hosts, have devised a number of mechanisms to manipulate their environment (within the body of the host) to their advantage. These include alterations in the physiology, and in some cases the behavior (Horton and Moore, 1993; Moore, 1995; Hurd, 1990), of the host. Among insects, the neural mechanisms underlying a manipulation by a parasite of the behavior of its host have been examined in only a few cases (Fouad et al., 1994, 1996; Adamo et al., 1997). A study of the mechanisms used in a particular parasite–host relationship can give us insights into the way that host behavior is controlled under more normal situations.

The parasitic braconid wasp *Cotesia congregata* lays its eggs inside the body of its host, larvae of the moth *Manduca sexta*. The *C. congregata* larvae develop within the hemocoel of their host, absorbing nutrients from the hemolymph, but they do not appear physically to damage the internal organs of the host. At the end of their second instar, the *C. congregata* larvae emerge from their host by burrowing through its cuticle. As they do this, the parasites molt to their third and final larval instar, then spin cocoons and pupate on the outer surface of the host. They remain attached to the outside of the body of the host, and the adult wasps emerge 4–5 days later (Beckage and Riddiford, 1978; Beckage and Templeton, 1986).

During the time that the *C. congregata* larvae are developing within their host, the parasitized *M. sexta* larvae grow more slowly than controls (Beckage and Riddiford, 1978, 1983; Alleyne and Beckage, 1997). However, there is little or no evidence of any parasite-induced changes in the behavior of the host until 8–12 h prior to the emergence of the parasites (Adamo et al., 1997). At this time, there is a dramatic change in the the behavior of the host, with a steep decline in spontaneous activity and in the amount of time spent feeding. The host remains in this state until its death, approximately 2 weeks later.

In a recent study, Adamo et al. (1997) found that the octopamine titer of host hemolymph was elevated 6.5-fold, from 22.2 to 143.7 pg μl^{-1}, at the time that these behavioral

changes began to occur. Because octopamine has been shown to be an important modulator of neural function and behavior in insects (Long and Murdock, 1983; Kinnamon et al., 1984; Ramirez and Orchard, 1990; Goldstein and Camhi, 1991; Ramirez and Pearson, 1991; Orchard et al., 1993; Weisel-Eichler and Libersat, 1996), we set out to examine whether this change in hemolymph octopamine titers could have an effect on nervous system function at around the time that the parasite emerges from its host. Earlier studies have shown that feeding can be suppressed by elevated levels of octopamine in the hemolymph (Ismail and Matsumura, 1992; Adamo et al., 1997), but the neural mechanisms underlying the changes in feeding behavior induced by this biogenic amine have remained unexplored.

Here, we have focused on the effects of parasitism by *Cotesia congregata* and of hemolymph octopamine levels on one aspect of feeding behavior, ingestion, in *Manduca sexta*. Ingestion involves the peristaltic movements of the foregut that are driven by the neurons of the frontal ganglion. The frontal ganglion is a small ganglion, 160 μm in diameter, that contains only approximately 35 neurons and is both necessary and sufficient to drive the activity of the foregut (Miles and Booker, 1994). We demonstrate that the motor pattern produced by the frontal ganglion is substantially altered at the time that the *C. congregata* larvae emerge from their host. We also provide evidence that this change in foregut function is due to the elevation in hemolymph levels of octopamine that coincides with the time of parasite emergence. We consider the consequences of a change in the activity of the foregut on both the behavior of the host and the survival strategies of the parasites.

Materials and methods

Animals

Larvae of *Manduca sexta* were obtained from our colony, where they were raised in individual containers under a 16 h:8 h L:D photoperiod at 27 °C. They were fed a wheatgerm-based artificial diet (Bell and Joachim, 1976). Third-instar larvae were parasitized by exposing them to a crowded colony of *Cotesia congregata*. They were then returned to their rearing containers until the parasites began to emerge, when they were moved to clean containers with fresh food. 24 h after the parasites emerged, their cocoons were collected from the hosts and placed into the *C. congregata* colony.

Cotesia congregata were obtained from Dr N. Beckage at the University of California, Riverside. They were raised in crowded conditions under a 16 h:8 h L:D photoperiod at 27 °C and fed a 3:1 honey:water solution.

Behavioral studies

Feeding behavior was assessed for normal and parasitized larvae. All larvae weighed between 2 and 3.8 g. Each larva was held in a separate container without food for 2 h, after which it was placed on its own leaf of *Nicotiana alata*. The larvae were left undisturbed for 2 h while their behavior was monitored by continuous observation. The *Nicotiana alata* leaves used were all of comparable age and size. For each minute of the test period, the behavior of the animals was assigned to one of the four following categories. (i) Eating: animals that were eating bit off pieces of leaf in a rhythmic feeding pattern. A feeding bout was determined to have ended when at least 2 min had elapsed before eating resumed. (ii) Tasting: tasting behavior consisted of animals repeatedly touching their mouthparts to a surface, without biting it. (iii) Moving: crawling or waving the anterior one-third to half of the body was categorized as moving. (iv) Quiescent: animals that were motionless were classified as quiescent. The percentage of the 2 h test period spent on each activity was determined from the sum of the time (in minutes) for which each animal exhibited each type of behavior. The amount of food consumed, if any, was determined as the area of leaf consumed at the end of the test period. To assess the efficiency of feeding behavior of the animals, these data are presented as the leaf area consumed per minute spent feeding for the entire test period. Bite rate was determined during a feeding bout from the number of bites of leaf taken in 30 s. All data are presented as the mean ±95 % confidence intervals (CIs) on the mean. Data are considered to be significantly different when the 95 % CIs do not overlap.

To test the role of the frontal ganglion in feeding behavior, it was removed from 10 normal fifth-instar larvae. The animals were anesthetized with CO_2, then placed in a chamber that provided a steady supply of CO_2 to the tracheae but left the head capsule exposed for surgery. All dissecting tools were soaked in 95 % ethanol before use and between animals. The head capsule was cleaned with 95 % ethanol, and an incision was made along the two lateral edges of the clypeus using a razor blade. The clypeus was folded down, exposing the frontal ganglion, which was either removed (for the experimental larvae) or touched with the dissecting tools for the sham-operated control larvae (N=10). A few crystals of phenylthoicarbamate (PTC, Sigma) were placed in the head capsule near the opening, the clypeus was folded back into place, and the wound was sealed with dermatological glue (New-Skin, Medtech Labs, Inc.). The larvae remained exposed to CO_2 until the glue had dried, when they were placed into clean individual containers with fresh diet. Behavioral tests were carried out the following day.

We have found that foregut movements are disrupted in parasitized larvae 8–12 h before parasite emergence, coincident with the decline in feeding behavior (C. I. Miles and S. A. Adamo, personal observation). Because this timing is somewhat variable and it can be difficult to predict when the parasites will emerge, our recordings and behavioral measurements were made 24 h after the parasite larvae began to emerge. To assess the behavior patterns and foregut activity of parasitized larvae before emergence of the parasites, we used parasitized larvae 2–4 days before the parasites were expected to emerge.

Electrophysiological recordings

Larvae were prepared for electrophysiological recordings as

described in an earlier study (Miles and Booker, 1994). Briefly, larvae are anesthetized with CO_2 or by chilling, then cut at the thoraco-abdominal junction. The abdomen is discarded, and the head and thorax are cut mid-dorsally, opened and pinned to a Sylgard-lined (Dow-Corning) dissection dish. This procedure exposes the foregut, part of the midgut, the brain and the frontal ganglion. In some cases, the foregut was recorded in this semi-isolated preparation. To isolate the frontal ganglion for the application of blood or other solutions, a small piece of parafilm was placed beneath the frontal ganglion, and the walls of a fluid-tight well were built around it with petroleum jelly. In many cases, however, we used an *in vitro* preparation in which the foregut, brain and frontal ganglion were dissected from the animal and placed in a well within a Sylgard-lined dish. Fluid-tight walls of petroleum jelly were constructed around the frontal ganglion to isolate it.

We found no apparent differences between the motor patterns of foreguts that remained in the body and those examined *in vitro*. In both preparations, we recorded the activity of the foregut muscles using either suction or hook electodes. The signal was amplified (A-M Systems), recorded on a video recorder (Vetter Instruments) and played back in real time using a high-speed chart recorder (Astro-Med Inc.). The most consistent change in foregut activity in the host larvae after parasite emergence was an increase in the burst period of the foregut muscles. This change was also apparent in observations of the movements of the foregut, which showed either dramatic increases in the period of peristalsis or a complete lack of peristalsis, which we classified as unpatterned activity. We therefore used the period for bursting activity of the esophageal constrictor muscles as the primary measure of changes in foregut activity. The period was measured from the chart recorder display, and the mean ±95 % CI on the mean was determined for 10 consecutive bursts. Differences were considered significant when the 95 % CIs did not overlap. To determine the mean period for all animals for a particular manipulation, data from 10 consecutive bursts from each animal were pooled and presented as means ±95 % CI. The same method of analysis was used to determine the number of spikes per burst and spike frequencies within bursts.

Application of blood and/or drugs

Blood was extracted from normal or parasitized fifth-instar larvae by anesthetizing the larva and then snipping an abdominal proleg near its base with scissors. The blood was collected in an Eppendorf tube on ice. A few crystals of PTC were added to the tube to prevent oxidation, and the mixture was vortexed and centrifuged to separate the solids. The supernatant was used for application to the frontal ganglion or foregut. The octopamine blockers mianserin or phentolamine (Sigma) were dissolved in physiological saline (Trimmer and Weeks, 1989) and diluted to a concentration 10-fold higher than that to be tested. This was then diluted 10-fold with the blood. For applications of blood alone, the blood sample was diluted tenfold with saline. D,L-Octopamine (Sigma or ICN Biomedicals Inc.) was dissolved and diluted in physiological saline. Foregut activity was recorded for 10 min before directly applying 20 µl of blood and/or drugs, during the application of drugs and for 10 min after application. The preparation was washed with saline, and recordings were made for 10 min before applying any additional treatment(s).

Results

Effects of parasitism on feeding behavior and the foregut

We assessed several aspects of the feeding behavior of parasitized larvae before (pre-emergence; N=11) and after (post-emergence; N=28) the emergence of their parasites. Compared with normal fifth-instar larvae in the same mass range (N=14), parasitized larvae prior to parasite emergence showed no significant differences in the average amount of time they spent eating, quiescent, moving or tasting (Fig. 1). After parasite emergence, these larvae showed a dramatic decline in the proportion of time they spent feeding (Fig. 1A). The pre-emergence parasitized larvae spent an average of 20.4±5.7 % of the 2 h test period eating, which is not significantly different from the behavior of unparasitized fifth-instar larvae (19.8±4.2 %). 24 h after emergence of the parasites, 11 of the 28 parasitized larvae tested failed to feed. For the 17 larvae that did feed, the average proportion of time they spent eating during the test period was 8.3±2.6 %. The data shown in Fig. 1A include values for the larvae that did not feed, and the average for this whole group is 5.0±2.3 % of the 2 h test period spent eating.

Post-emergence larvae showed a significant increase in the proportion of the test period spent quiescent (Fig. 1B). This increase was primarily at the expense of the amount of time spent eating. Although these larvae spent less than half the amount of time moving compared with pre-emergence or unparasitized larvae, this difference was not significant (Fig. 1C). There was also no significant difference in the proportion of time they spent exhibiting tasting behavior (Fig. 1D). The post-emergence animals repeatedly touched their mouthparts to the food, but they usually failed to initiate a feeding bout.

The post-emergence parasitized larvae also showed a significant decline in their efficiency for ingesting food (Fig. 2A). The area of leaf consumed averaged $0.2\pm0.07\,cm^2\,min^{-1}$ spent feeding for unparasitized larvae and $0.19\pm0.05\,cm^2\,min^{-1}$ spent feeding for pre-emergence parasitized larvae. Following the emergence of the parasites, the parasitized larvae that ate consumed only $0.059\pm0.018\,cm^2\,min^{-1}$ spent feeding. This reduction in the amount of food consumed was not due to a lower bite rate during the feeding bout, because this was not significantly different between pre- and post-emergence larvae (1.35 ± 0.08 bites min^{-1} for pre-emergence larvae, N=10, and 1.2 ± 0.1 bites min^{-1} for post-emergence larvae, N=10). Instead, the reduction in the rate of consumption appeared to be due to a decline in the ability of the larva to ingest the food.

The frontal ganglion and the foregut it controls are important in the ingestion of food, so we examined the effects

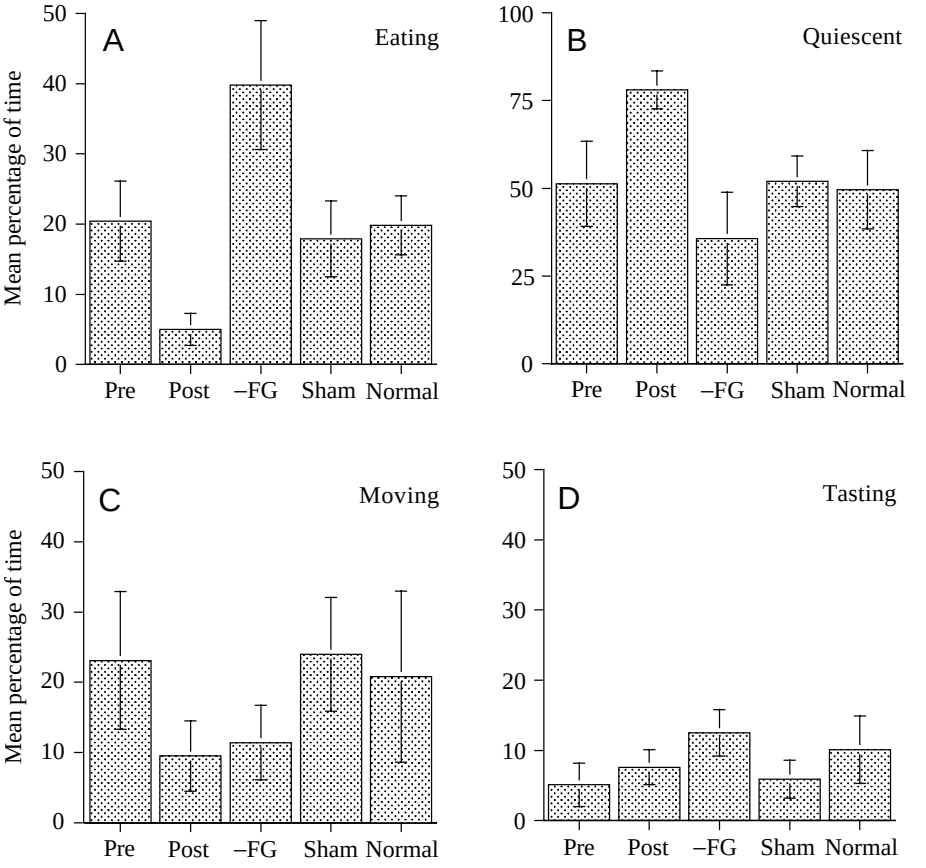

Fig. 1. Behavior of fifth-instar larvae after various treatments: mean percentage of time during a 2 h test period the animals spent exhibiting each of the four types of behavior. Definitions of each type of behavior are given in the text. 100 % of the 2 h test period is accounted for by these four behavioral categories. Pre, parasitized larva 2–4 days before parasite emergence (N=11); Post, parasitized larvae 24 h after parasite emergence (N=28); –FG, unparasitized fifth-instar larvae 24 h after removal of the frontal ganglion (N=10); Sham, sham-operated control fifth-instar larvae, 24 h after the operation (N=10); Normal, unoperated unparasitized fifth-instar larvae (N=14). Values are means ± 95 % CI.

of removing the frontal ganglion on the feeding behavior and rate of food consumption of the larvae. Sham-operated control larvae (N=10) showed no significant differences in the proportion of time spent feeding, resting, tasting or moving compared with normal fifth-instar larvae or pre-emergence parasitized larvae (Fig. 1A–D). The larvae lacking a frontal ganglion, however, spent an average of 39.8±9.2 % (N=10) (Fig. 1A) of the test period eating, which is nearly twice as much time as normal, sham-operated or pre-emergence parasitized larvae. Their rate of food consumption, however, was greatly reduced over that of normal, sham-operated or pre-emergence parasitized larvae. Larvae without a frontal ganglion consumed the food at a rate of $0.078\pm0.023\,cm^2\,min^{-1}$ spent feeding, a value that is not significantly different from that observed in post-emergence parasitized larvae (Fig. 2A).

We also examined the effect of frontal ganglion removal and parasitism on the number of feeding bouts and their duration. Although parasite emergence and removal of the frontal ganglion both had significant, although opposite, effects on the total amount of time the animals spent feeding, these effects were not due to changes in the average duration of their feeding bouts (Fig. 2B). Normal larvae exhibited feeding bouts with mean durations of 5.34±1.34 min. Feeding bouts for post-emergence parasitized larvae had mean durations of 6.14±1.61 min, while larvae with their frontal ganglion removed had feeding bouts with mean durations of 6.87±1.24 min. Thus, the durations of the bouts were not significantly changed. Parasite emergence and removal of the frontal ganglion did, however, have significant effects on the average number of feeding bouts initiated by these animals (Fig. 2C). Normal control larvae initiated 3.9±0.8 feeding bouts during a 2 h test period. In contrast, in those post-emergence parasitized larvae that did eat, an average of only 1.2±0.2 feeding bouts were initiated, which is significantly lower than for controls. Larvae without a frontal ganglion initiated 6.3±1.2 feeding bouts, a value significantly higher than for controls. Both parasitism and removal of the frontal ganglion therefore changed the proportion of time a larva spent feeding by altering the frequency with which feeding bouts were initiated.

Changes in foregut activity in post-emergence larvae

Previously, we established that the foregut of both larval and adult *M. sexta* plays a central role in the ingestion of food (Miles and Booker, 1994, 1998). The reduced rates of food consumption by larvae with their frontal ganglion removed and by post-emergence parasitized larvae suggested that the decline in ingestion rate was due to changes in the activity of the frontal ganglion and foregut. We therefore compared the peristaltic activities of the foregut of control and parasitized larvae. The foregut of normal and pre-emergence parasitized larvae showed posteriorly directed peristalsis that was maintained for several hours in isolated preparations (Fig. 3A,B). The motor pattern for this activity has been described for normal larvae in an earlier study (Miles and

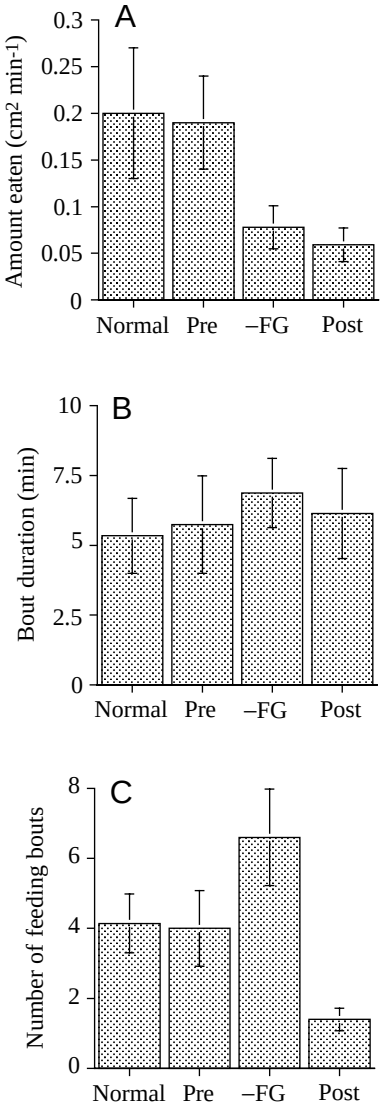

Fig. 2. Effects of removal of the frontal ganglion and parasite emergence on two aspects of feeding behavior. Mean values, with error bars denoting the 95 % CI on the mean, are shown. (A) The rate of ingestion is shown for unparasitized, unoperated fifth-instar larvae (Normal) (*N*=10), parasitized larvae 2–4 days before parasite emergence (Pre) (*N*=10), fifth-instar larvae 24 h after removal of the frontal ganglion (–FG) (*N*=10) and parasitized larvae 24 h after emergence of the parasites (Post) (*N*=10). (B) Durations of feeding bouts for the same larvae as in A. (C) The number of feeding bouts initiated in a 2 h period by these individuals.

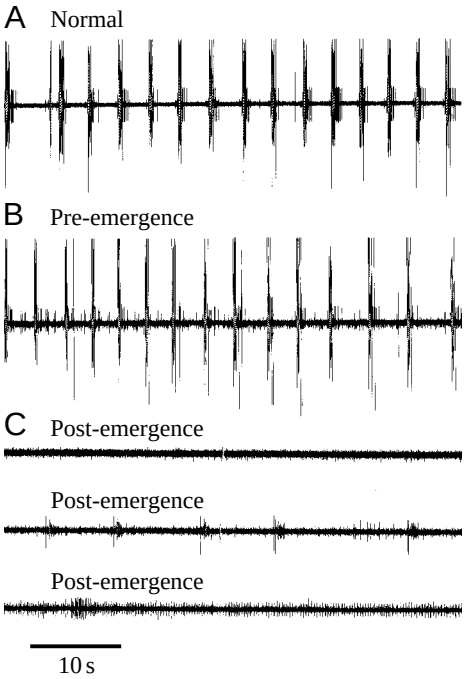

Fig. 3. Activity of the esophageal musculature in (A) an unparasitized fifth-instar larva and (B) a parasitized larva 4 days before the emergence of the parasites. In both larvae, the foregut displayed robust peristalsis. The large-amplitude bursts are from the esophageal constrictor muscles. In B, the activities of the esophageal dilator muscles are also visible as the small-amplitude spikes. (C) Examples of foregut muscle activity in three post-emergence parasitized larvae, 24 h after parasite emergence. In the top of the three traces, the foregut displayed only weak tremors. The foregut of the animal shown in the middle trace showed weak peristalsis; bursts are from the esophageal constrictor muscles. The foregut in the bottom trace showed one weak constriction of the esophagus, evident here as a weak burst of the esophageal constrictor near the start of the recording.

Booker, 1994), which included an analysis of the phasing of constrictor and dilator bursts. It was found that shifts of up to one-quarter of a cycle produced no discernible changes in the peristaltic activity of the foregut measured using video analysis. The period of bursting in the esophageal constrictor muscles of pre-emergence parasitized larvae (3.3±0.6 s, *N*=8) was not significantly different from that of the normal larvae (3.2±0.1 s, *N*=109) (Figs 3A,B, 4). Similarly, the number of spikes per burst (9.3±0.5 spikes s^{-1} for pre-emergence parasitized larvae and 9.5±0.4 spikes s^{-1} for normal larvae) was not significantly different, while the spike frequencies in each burst (26.0±1.7 for pre-emergence parasitized larvae and 20.8±1.0 for normal larvae) were actually significantly higher for the foreguts of parasitized larvae before the emergence of the parasites.

Post-emergence parasitized larvae (*N*=18) showed a range of disruptions of foregut activity (Figs 3C, 4). Peristaltic activity of the foregut was never observed in 10 of these larvae; the foregut of these animals exhibited only weak tremors, which were reflected in electrophysiological recordings as unpatterned spike activity (Fig. 3C). The foregut of the remaining eight larvae did exhibit a few peristaltic waves, but these movements were weak, with significantly lower numbers of spikes per burst (5.9±0.7) (Fig. 4B) and lower spike frequencies within the bursts (7.4±1.1 spikes s^{-1}) (Fig. 4C). In addition, the period of burst activity was significantly longer, with a mean value of 9.4 s, and more variable, as indicated by the wider 95 % confidence intervals of ±1.97 s (Fig. 4A). Frequent rinsing of the foregut of these larvae did not revive their activity, even if they were placed *in vitro.*

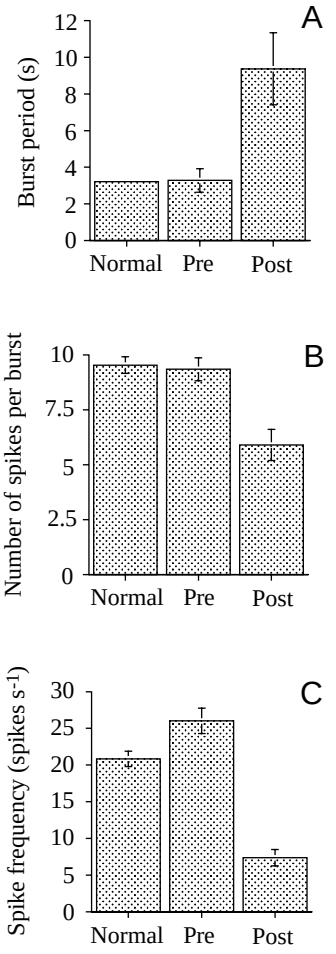

Fig. 4. Foregut activity in unparasitized control larvae (Normal, *N*=109), in parasitized larvae 2–4 days before parasite emergence (Pre, *N*=8) and in parasitized larvae 24 h after parasite emergence (Post, *N*=8). In addition to the eight individuals included in the post-emergence group, an additional 10 animals showed no rhythmic foregut activity. (A) Mean burst period for esophageal peristalsis. Error bars denote 95 % CI on the mean; values are given in the text; for the control, these were too small for the scale of the plot. (B) Number of spikes per burst for each group. (C) Spike frequency for the bursts.

Application of parasitized blood

All the effects of parasitized blood on the foregut motor pattern described here appear to be mediated by the frontal ganglion because the application of blood and/or drugs to the frontal ganglion alone is both necessary and sufficient to produce them. Application to the foregut musculature alone was ineffective. Applying blood from post-emergence parasitized larvae to the frontal ganglion of normal feeding larvae disrupted normal foregut activity (Fig. 5, *N*=25). For eight individuals, the foregut activity was completely disrupted, resulting in only weak tremors, recorded electrophysiologically in the foregut as unpatterned spiking. The remaining larvae, which maintained peristalsis after the application of parasitized blood, showed both a significant decrease in the number of spikes per burst in the esophageal

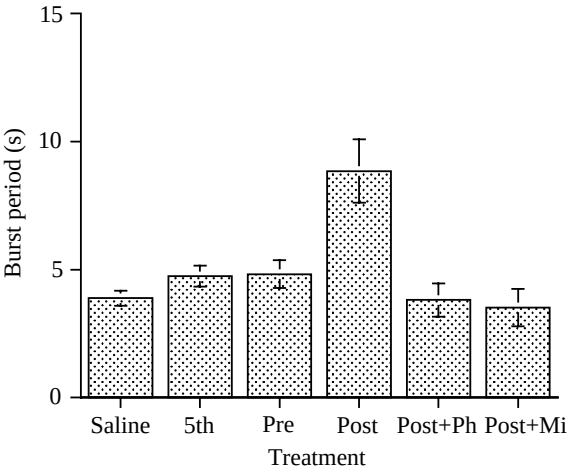

Fig. 5. Effects of application of blood to the frontal ganglion on the mean burst period of esophageal muscles in normal fifth-instar larvae. Error bars denote 95 % CI on the mean. Saline, mean burst period for the esophageal muscles before blood application (*N*=22). 5th, mean burst period 3 min after application of blood from normal, unparasitized fifth-instar larvae (*N*=15). Pre, mean burst period 3 min after application of blood from pre-emergence parasitized larvae (*N*=15); Post, mean burst period 3 min after application of blood from post-emergence parasitized larvae (*N*=17). Also shown are the effects of co-application of post-emergence parasitized blood and 10^{-5} mol l^{-1} phentolamine (*N*=8; Post+Ph) and of post-emergence blood and 10^{-7} mol l^{-1} mianserin (*N*=5; Post+Mi).

musculature, from 8.9±0.5 before application to 4.7±0.4 after application, and a significant decline in spike frequency within the bursts, from 20.4±2.1 spikes s^{-1} before application to 14.8±1.9 spikes s^{-1} after application. In addition, they showed significant increases in the period of this peristaltic activity and a decrease in its regularity that was reflected in the wider range of the 95 % confidence intervals. The mean period for the esophageal constrictor bursts increased from 2.7±0.1 s before the application of parasitized blood to 8.8±1.2 s after the blood had been applied (Fig. 5), a value that was not significantly different from that of post-emergence parasitized larvae. When peristaltic activity remained, the phasing of the activities of the dilator and constrictor muscles relative to one another was not affected by the post-emergence parasitized blood. The effect of the parasitized blood was readily washed out (see Fig. 8A).

Blood from either normal fifth-instar larvae or pre-emergence larvae typically caused no significant change in the period of peristalsis in normal fifth-instar larvae when applied to the frontal ganglion (Fig. 5). These animals showed an average burst period of 3.9±0.3 s (*N*=37) before the application of either type of blood. The larvae given fifth-instar blood showed an average burst period of 4.7±0.5 s (*N*=15) after the blood had been applied to the frontal ganglion, and those given pre-emergence parasitized larval blood had an average burst period of 4.8±0.6 s (*N*=15) after it had been applied. Similarly, within a burst, the spike frequency and number of spikes per burst did not change significantly upon application of fifth-instar or pre-emergence parasitized blood (data not shown).

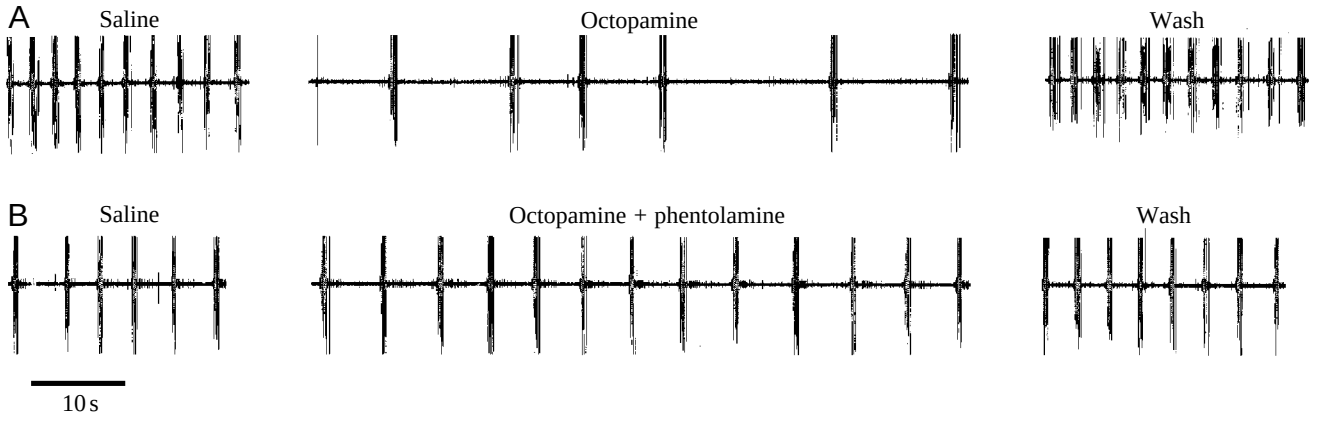

Fig. 6. (A) Application of 10^{-6} mol l^{-1} octopamine to the frontal ganglion disrupts foregut activity in a normal fifth-instar larva. Recordings show bursts from the esophageal constrictor muscles. (B) This effect can be blocked by co-application of 10^{-5} mol l^{-1} phentolamine. All data are from the same individual. For both A and B, the left-hand panel shows the activity of the foregut before the application of the blood, the middle panel shows the activity 3 min after application, and the right-hand panel shows the activity 30 s after washing with saline.

Among the larvae given blood from normal fifth-instar larvae, an additional two individuals exhibited disrupted foregut activity, resulting in weak tremors. The same result was obtained for an additional five animals given blood from pre-emergence parasitized larvae. Thus, while application of normal fifth-instar or pre-emergence parasitized larval blood to the frontal ganglion could disrupt the activity of the foregut, this effect was less commonly observed than after application of post-emergence parasitized blood. In most cases, application of normal fifth-instar or pre-emergence parasitized blood had no significant effect.

Effects of octopamine

It has been shown that at around the time of parasite emergence there is an approximately 6.5-fold increase in the circulating titer of octopamine in the hemolymph (Adamo et al., 1997). In pre-emergence parasitized larvae, the level of octopamine in the hemolymph was reported to be 22.2 pg μl^{-1} (1.2×10^{-7} mol l^{-1}), which increases to 143.7 pg μl^{-1} (7.6×10^{-7} mol l^{-1}) following emergence of the parasites. This result suggests that octopamine is a potential candidate for mediating some of the changes in foregut activity in the host at the time of parasite emergence. We tested this possibility by monitoring the responses of the foregut of unparasitized larvae to the application of octopamine to the frontal ganglion alone. Application of 10^{-6} mol l^{-1} octopamine to the frontal ganglion of normal fifth-instar larvae caused complete disruption of foregut activity in eight out of 32 individuals, with the remainder showing a significant increase in peristalsis period from 3.3±0.3 s before application of octopamine to 6.9±0.9 s after application (Figs 6A, 7). This value is not significantly different from the burst periods of post-emergence parasitized larvae or normal larvae treated with post-emergence parasitized blood. At higher octopamine concentrations, such as 10^{-5} mol l^{-1}, the mean period is lengthened further (Fig. 7). Consistent with a weakening of the peristalsis observed upon application of octopamine, treating the frontal ganglia with

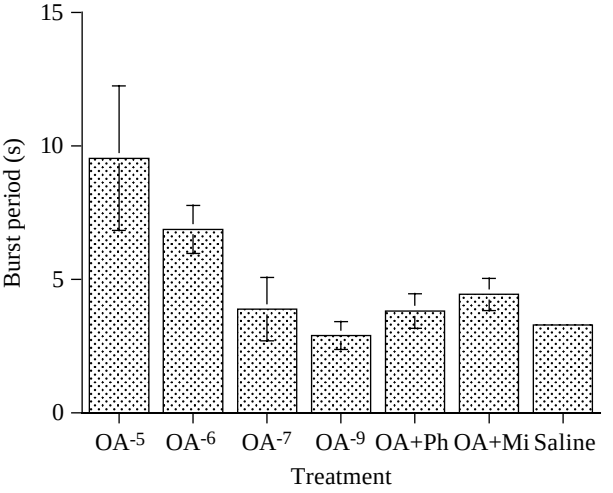

Fig. 7. Octopamine applied to the frontal ganglion (FG) at concentrations of 10^{-6} mol l^{-1} or higher significantly increases the mean burst period for foregut muscle activity. Error bars denote 95 % CI on the mean, with values given in the text; for the saline animal, this was too small for the scale of the plot. Saline, mean burst period before application of the octopamine solutions (N=47). OA^{-5}, mean burst period 3 min after the application of 10^{-5} mol l^{-1} octopamine (N=6). A further two individuals showed a complete disruption of foregut peristalsis. OA^{-6}, mean burst period 3 min after application of 10^{-6} mol l^{-1} octopamine (N=24). OA^{-7}, mean burst period 3 min after application of 10^{-7} mol l^{-1} octopamine (N=8). OA^{-9}, mean burst period 3 min after application of 10^{-9} mol l^{-1} octopamine (N=6). OA+Ph, mean burst period 3 min after co-application of 10^{-6} mol l^{-1} octopamine and 10^{-5} mol l^{-1} phentolamine (N=5). OA+Mi, mean burst period 3 min after co-application of 10^{-6} mol l^{-1} octopamine and 10^{-7} mol l^{-1} mianserin (N=5).

octopamine also resulted in a decline in the number of spikes per burst, from 10±0.51 before application to 7.2±0.4 after application, and a decrease in spike frequency within the bursts from an initial value of 21.1±1.1 spikes s^{-1} to 17.8±1.2 spikes s^{-1} after application of octopamine. The

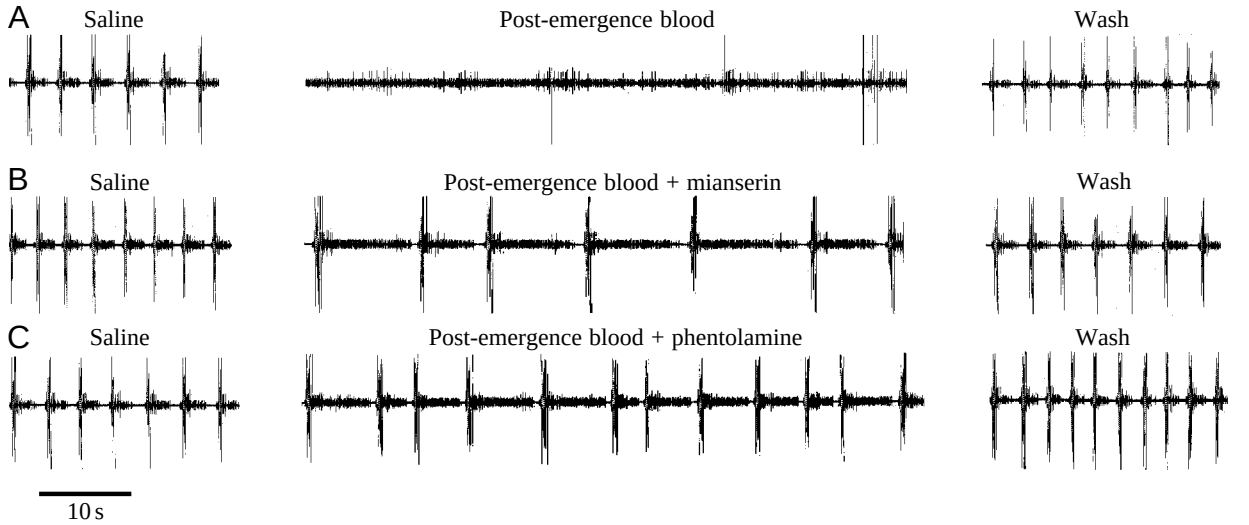

Fig. 8. The effects of applying parasitized blood to the frontal ganglion can be partially blocked by phentolamine or mianserin. All recordings are from the same individual. Large-amplitude bursts are from the esophageal constrictor muscles; smaller-amplitude, longer-duration bursts are from the esophageal dilators. For A, B and C, the left-hand panel is the activity of the foregut before the application of the blood, the middle panel is the activity 3 min after application, and the right-hand panel is the activity 30 s after washing with saline. (A) Application of blood from a post-emergence parasitized larva disrupts the activity of the foregut musculature in a normal fifth-instar larva. (B) Co-application of 10^{-8} mol l^{-1} mianserin prevents the total disruption of the foregut motor pattern, although there is an increase in period. (C) Co-application of 10^{-5} mol l^{-1} phentolamine blocks the effects of parasitized blood.

phasings of the dilators and constrictors within a peristaltic burst were not affected (not shown). Disruption of foregut activity by 10^{-6} mol l^{-1} octopamine could be blocked by 10^{-5} mol l^{-1} phentolamine (N=5; Figs 6B, 7) or 10^{-7} mol l^{-1} mianserin (N=5; Fig. 7). Octopamine was ineffective when applied to the foregut musculature alone.

Blocking the effects of parasitized blood

To confirm a role for octopamine in disrupting foregut activity in the post-emergence parasitized larvae, we tested whether phentolamine or mianserin could block the ability of blood from post-emergence parasitized larvae to disrupt the peristaltic activity of the foregut. This disruption could be prevented by phentolamine at 10^{-5} mol l^{-1} (N=8), or by 10^{-7} mol l^{-1} mianserin (N=4; Figs 5, 8). Both treatments resulted in a significant decrease in the burst period compared with the application of post-emergence blood alone. Decreases in spike number and frequency within the bursts were also prevented by treating the blood with these concentrations of phentolamine and mianserin (Fig. 8B,C).

Discussion

Changes in feeding behavior following parasite emergence

Our observation that there is a decline in the proportion of time that post-emergence parasitized larvae spend feeding and an increase in the proportion of time spent in a quiescent state is consistent with data from earlier studies (Beckage and Riddiford, 1978, 1983; Adamo et al., 1997). In the present study, the increase in time devoted to quiescent behavior appeared to be at the expense of the time spent eating because there was no significant difference in the amount of time spent moving or tasting. It was somewhat surprising that these larvae did not show a significant decline in the proportion of time spent moving, as has been reported in the other studies. This may have been due to a number of factors, such as the relatively short time that elapsed between parasite emergence and the behavioral examination (24 h), as well as to the use of different criteria for assessing movement. For example, Adamo et al. (1997) measured the distance crawled by larvae placed in an empty arena. We assessed movement in larvae placed on a plant which, although a more natural situation, may lead to reduced levels of movement by the larvae since they are continuously in contact with their food. Perhaps because of this, our behavioral results showed a wide variation in the proportion of time that even the unoperated control larvae spent moving. Therefore, while the average for post-emergence larvae appeared to be lower, it did not differ significantly from that of the other groups.

It is also of interest that the post-emergence larvae did not exhibit a decline in the activity we defined as tasting, in which the larvae repeatedly touch the chemosensory sensilla on their mouthparts to the surface of the food. This behavior has been described as a component of feeding behavior that typically leads to the initiation of a feeding bout (Bowden, 1988; Bowden and Wyse, 1997; Glendinning et al., 1998). Our results indicate that appetitive behavior in the post-emergence parasitized larvae is not disrupted. Instead, it is the trigger for initiating the feeding bout that is altered after the parasites emerge.

Post-emergence parasitized larvae showed significant deficiencies in their ability to ingest food. If the amount of leaf

area consumed per minute spent feeding is compared between post-emergence larvae and pre-emergence parasitized or unparasitized larvae, the post-emergence larvae consume less than half as much food per unit time. This is not due to a reduction in the frequency of biting in these larvae, because the bite rate of post-emergence larvae is not significantly different from that of pre-emergence larvae. Instead, the post-emergence larvae appear to experience difficulty in biting off pieces of food and ingesting them.

Activity of the foregut is driven by the neurons of the frontal ganglion (Miles and Booker, 1994). If foregut movements are disrupted by interfering with the motor pattern produced by the frontal ganglion, the ability of the larva to ingest food will be compromised. This was demonstrated by removing the frontal ganglion from normal fifth-instar larvae, and measuring the amount of leaf area consumed per unit time of feeding. The values for rate of consumption in the operated and post-emergence parasitized larvae were not significantly different, suggesting that a disruption in foregut peristalsis in the post-emergence larvae may be responsible for their reduced efficiency at ingesting food.

Our observations of the feeding behavior of post-emergence parasitized larvae and larvae without a frontal ganglion provide some insight into the regulation of feeding behavior in normal *M. sexta*. They indicate that feeding behavior consists of at least four components that are subject to different control mechanisms in the nervous system: (i) tasting, (ii) initiation of the feeding bout, (iii) ingestion, and (iv) termination of the feeding bout. Parasite emergence has no effect on the amount of time spent tasting the food. In addition, bout durations appear to be normal in these animals, indicating that termination of the bout has also not been affected. However, the emergence of its parasites triggers a dramatic suppression of the tendency of a host to initiate feeding bouts within a 2 h period. Post-emergence parasitized larvae initiated fewer than one-third as many feeding bouts as normal larvae. As discussed above, these larvae also showed a significant reduction in their abilitiy to ingest food once a feeding bout had been initiated as a result of changes in the activity of the frontal ganglion. It is possible that the disruption of ingestion may be a contributing factor to the reduction in the number of feeding bouts initiated by post-emergence larvae. Our results from larvae with their frontal ganglion removed indicate, however, that this is not the case, since these larvae spent twice as much time eating as sham-operated or normal controls. This increase in feeding time was due to an increase in the number of feeding bouts initiated, not because feeding bouts lasted significantly longer. As in post-emergence parasitized larvae, the termination of the feeding bout was not affected by removal of the frontal ganglion. In both cases, the larvae ingested substantially less than normal during a feeding bout, yet they did not compensate for this by increasing the length of the bout. This indicates that, in *M. sexta*, feeding bouts are probably not terminated by volumetric feedback from foregut stretch receptors as in flies and locusts (Gelperin, 1971; Simpson, 1983), a view consistent with the conclusions of Timmins and Reynolds (1992). Our results are also consistent with those of Reynolds et al. (1986), who found that larvae within the range of masses we used in these experiments (2–3.8 g) ate more by increasing bout frequency not bout duration. Timmins and Reynolds (1992) provided evidence that feedback concerning the level of nutrients ingested regulates the duration of a feeding bout. While we did not test this hypothesis directly, our data do not support it, since all the larvae were fed leaves of similar age, size and, presumably, nutrient content. Despite dramatic differences in the area of leaf consumed by normal *versus* post-emergence larvae or those without their frontal ganglion, their feeding bouts did not differ significantly in length. Our results suggest that, at least within the size range 2–3.8 g, *M. sexta* larvae have feeding bouts with set durations, and they regulate the amount they consume by varying the number of times they initiate feeding bouts.

Foregut activity in parasitized larvae

In normal larvae, the foregut is continuously active, typically showing posteriorly-directed peristalsis (Miles and Booker, 1994). The pattern of foregut activity in parasitized larvae 2–4 days prior to the emergence of the parasites was indistinguishable from that of normal, unparasitized larvae. However, foregut activity in post-emergence larvae was dramatically reduced. The effect was variable, with foregut activity that ranged from slow weak peristalsis to unpatterned tremors. If the foregut and frontal ganglion of the parasitized larva were placed *in vitro*, even frequent rinsing with saline did not revive its activity.

Effects of parasitized blood on unparasitized frontal ganglia

Application of hemolymph from post-emergence parasitized larvae disrupted the ongoing peristaltic activity of the foregut of normal, feeding fifth-instar larvae. This effect was mediated by the neurons of the frontal ganglion because it was induced by application of hemolymph from post-emergence larvae onto the frontal ganglion alone and not by application to the foregut musculature. The factor in the blood of post-emergence parasitized larvae appeared to be unique to these larvae since application of hemolymph from normal fifth-instar or parasitized larvae that were still feeding did not disrupt foregut activity in most cases. In a few cases, blood from the normal fifth-instar larvae or pre-emergence parasitized larvae did disrupt foregut activity. Octopamine levels in insect hemolymph are known to be elevated in response to stress (Davenport and Evans, 1984), and it is possible that the stress of collecting the blood samples from the donors was sufficient to elevate their hemolymph octopamine levels, despite our attempts to minimize it.

The activity of normal foreguts treated with post-emergence parasitized hemolymph resembled that observed in post-emergence parasitized larvae, with lengthened periods and lower spike numbers and frequencies within bursts, or a total lack of bursting, resulting in unpatterned spiking. Unlike the case for post-emergence parasitized larvae, the effect of the application of hemolymph from such larvae on the normal

larval foregut is not permanent, but can be washed out. This difference could be related to the fact that the parasitized foregut had experienced a chronic exposure to the post-emergence hemolymph, which could result in permanent changes to components of the circuit. This possibility is supported by the observation that continuously exposing the frontal ganglion of a normal larva to post-emergence hemolymph for more than 30 min induced an irreversible breakdown of the peristaltic motor pattern (C. I. Miles, personal observation).

In nearly all cases, the foregut muscles did not show an alteration in their firing patterns relative to one another, i.e. if bursting occurred, the phasing of the bursts was the same as before application of the post-emergence hemolymph. In cases where bursting was completely abolished, both dilators and constrictors were affected. We were unable to find any consistent evidence that certain motoneurons of the peristalsis motor program were more susceptible to the effects of post-emergence hemolymph than others. The post-emergence hemolymph may therefore exert its effect on the foregut motor pattern by a general suppression of all motoneuron activity. Among the motoneurons so far identified, we have not yet observed any that appear to have endogenous bursting properties (Miles and Booker, 1994). An alternative (or concurrent) target of the post-emergence hemolymph could be an unidentified pacemaker component of the foregut circuitry, which could be an interneuron or a motoneuron. The neuronal basis for the changes in the frontal ganglion motor program that we have described in the present study remains to be determined.

The effect of octopamine on foregut activity

An earlier study reported that octopamine levels in the hemolymph of parasitized *M. sexta* larvae are elevated 6.5-fold at the time the larvae stop feeding (Adamo et al., 1997). We therefore tested the effects of applying octopamine to the frontal ganglion alone on the activity of the foregut of normal fifth-instar larvae. When 10^{-6} mol l^{-1} octopamine (approximately the concentration found in post-emergence parasitized hemolymph) was applied to the frontal ganglion of normal larvae, foregut activity was disrupted in a manner similar to that observed upon application of post-emergence parasitized hemolymph. This concentration of octopamine is approximately 1000 times lower than the concentration typically effective in modulating the activity of intact insect brains and segmental ganglia (Ramirez and Pearson, 1991; Goldstein and Camhi, 1991; Weisel-Eichler and Libersat, 1996). It is also 100 times lower than the hemolymph levels of octopamine that have been shown to suppress feeding behavior effectively (Ismail and Matsumura, 1992; Adamo et al., 1997). These higher concentrations of octopamine are believed to be necessary because the connective tissue sheath surrounding the brain and segmental ganglia effectively prevents this biogenic amine from reaching the neurons (Lane and Treherne, 1972; Stevenson, 1989). Our results suggest that the connective tissue sheath surrounding the frontal ganglion may be more permeable than that covering the brain and other central ganglia. The frontal ganglion may, therefore, be a target for a number of circulating hormones or modulators that are present in concentrations too low to affect the neurons of the central nervous system.

We tested the effectiveness of two known octopamine antagonists in blocking the disruptive effects of octopamine on the pattern of foregut motor activity. We found that 10^{-5} mol l^{-1} phentolamine was effective in blocking the disruptive effects of 10^{-6} mol l^{-1} octopamine, while mianserin was effective at 10^{-7} mol l^{-1}. The stronger effect of mianserin compared with phentolamine is consistent with the relative effectiveness of these antagonists on octopamine receptors in insect neural tissue and in the locust abdominal air sac (Roeder, 1991; Zeng et al., 1996).

Mianserin and phentolamine were also tested for their abilities to block the effects of parasitized blood on frontal ganglion activity. Mianserin effectively prevented disruption of the foregut motor pattern at 10^{-7} mol l^{-1}, while phentolamine was effective at 10^{-5} mol l^{-1}. Although both mianserin and phentolamine have proved to be very effective on insect octopamine receptors, they are not specific to octopamine. For example, phentolamine is an α-adrenergic receptor blocker in the vertebrate nervous system, and mianserin blocks 5-HT (serotonin) receptors. Thus, although our pharmacological study is consistent with octopamine being the active substance in post-emergence blood, effects of these compounds on other neurotransmitters or neuromodulators cannot be ruled out.

The post-emergence parasitized condition is a pathological one; however, octopamine is likely to be used under more normal conditions as well because it is a commonly used neurotransmitter, neuromodulator and/or neurohormone in insects (Long and Murdock, 1983; Kinnamon et al., 1984; Ramirez and Orchard, 1990; Goldstein and Camhi, 1991; Ramirez and Pearson, 1991; Orchard et al., 1993; Weisel-Eichler and Libersat, 1996). Octopamine-immunoreactive cell bodies have been observed in the frontal ganglion (K. A. Mesce, personal communication), so it is likely that this biogenic amine is normally used as a transmitter/modulator in the frontal ganglion. Octopamine levels in the hemolymph of insects may be elevated during times of stress or elevated activity, such as flight (Davenport and Evans, 1984; Goosey and Candy, 1980), and octopamine has been reported to serve a role analogous to adrenaline as a ‘flight or fright’ hormone (Orchard, 1982). A reduction in gut function induced by octopamine would be consistent with this analogy.

What benefits does a parasite gain by modulating the frontal ganglion of its host?

Successful development for the parasite *Cotesia congregata* is intimately linked to the growth and development of its host *Manduca sexta*. During its first two larval instars, the parasite is dependent on the growth and survival of its host to provide it with adequate nutrition for its own growth and development. Once they have molted to their final larval instar and emerged

from their host, the parasitic *C. congregata* remain dependent on *M. sexta* to provide an undisturbed surface for the next 4–5 days so that they can complete development before emerging from the cocoons as free-living adults. By dramatically altering the food intake of their hosts, the parasites can create such an environment for themselves. It has been suggested (Adamo et al., 1997) that the parasite benefits from suppressing feeding in its host at this stage because the cocoons risk being eaten by a feeding host. We propose that an added benefit to the parasite results from the close relationship between weight gain and the triggers for molting and metamorphosis in *M. sexta* larvae (Safranek and Williams, 1984a,b). Adamo (1998) showed that cocoons of *C. congregata* show the highest survival rates when they are attached to the surface of a living host. If the host were to undergo a molt, the cocoons could either be lost with the shed cuticle or, more likely, the host would be trapped in an unsuccessfully shed cuticle and die. By triggering a reduction in the number of feeding bouts as well as the efficiency with which food is ingested, the parasite can ensure that the host will not gain sufficient weight to initiate a molt (Nijhout, 1975). While it is possible for starved larvae to undergo a molt, this would not occur until well past the 4–5 days required for the wasps to complete adult development (Safranek and Williams, 1984b).

Parasites emerging from a fifth-instar *M. sexta* larva must further ensure that the host does not initiate metamorphosis before they complete their development. In normal fifth-instar *M. sexta* larvae, the onset of metamorphosis is marked by wandering behavior, when they burrow underground to pupate, and it is unlikely that the wasp cocoons could either survive the process of digging or be able to emerge from the underground pupation chamber of their host. Metamorphosis is triggered by a peak of ecdysone that occurs in the absence of juvenile hormone (JH). The decline in JH titer at the end of the fifth larval instar has been shown to be due in part to an increase in the levels of JH esterases (Sanburg et al., 1975), the release of which are triggered by larval mass (Cymborowski et al., 1982). In parasitized larvae, levels of JH esterases do not increase in the hemolymph, and in fact decline to almost zero by the third day of the instar (Beckage and Templeton, 1986). It is likely that the lack of weight gain by post-emergence larvae is at least partly responsible for this effect.

By inducing an elevation of the level of octopamine in the hemolymph of their hosts, the *C. congregata* larvae disrupt the function of the frontal ganglion of the host and the efficiency with which it can feed and gain weight. While it has been demonstrated that octopamine can lead to anorexia in *M. sexta* larvae, the levels required experimentally to produce this effect are so much higher than those observed in post-emergence larvae that it is not clear whether octopamine could be responsible for the dramatic reduction in the number of feeding bouts these larvae initiate. At this point, the clearest effect of the elevation of octopamine levels in post-emergence larvae is the reduced efficiency of ingestion due to the effects of octopamine on the frontal ganglion. It remains to be determined how *C. congregata* larvae cause the elevation of octopamine levels in post-emergence larvae. Recent measurements of the half-life of octopamine in the hemolymph of pre- and post-emergence larvae indicate that it is prolonged in post-emergence larvae, indicating a disruption in the ability of these larvae to break down octopamine in the hemolymph (Adamo et al., 1999). How this effect is produced by the larvae of *C. congregata* remains to be determined.

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