The Journal of Experimental Biology **198**, 1087–1092 (1995)
Printed in Great Britain © The Company of Biologists Limited 1995

THE FEEDING STIMULUS IN *RHODNIUS PROLIXUS* IS TRANSMITTED TO THE BRAIN BY A HUMORAL FACTOR

H. MULYE AND K. G. DAVEY*

Department of Biology, York University, North York, Ontario, Canada M3J 1P3

Accepted 4 January 1995

Summary

Neurosecretory cells in the brain of *Rhodnius prolixus* are known to be the source of an ovulation hormone released at feeding. They were selected to test the hypothesis that feeding brings about the release of another hormone in the abdomen which is transported forward to activate the neuroendocrine axis in the brain, and that severing the aorta interferes with this transport. These cells have previously been shown to exhibit an increase in action potential frequency at the time of release of their hormone. In normal females, the spike frequency of the cells increased after feeding and remained high over at least the next 24 h. In females with the dorsal vessel severed, the spike frequency remained low, at levels near those of unfed females, except for a transitory increase 4 h after feeding. The spike frequency of the neurosecretory cells in females with the dorsal vessel severed increased when hemolymph from fed normal females or from those with their dorsal vessel severed was placed directly on the brains of the test females. Hemolymph taken from unfed females did not produce this response. The activity in the hemolymph was heat-stable and disappeared after pronase or trypsin digestion. Similar activity was present in the fused thoracico-abdominal ganglionic mass, but absent from fore-, mid- and hindgut and from the abdominal neurosecretory organs.

Key words: neuropeptides, electrophysiology, neurosecretion, blood meal, feeding, *Rhodnius prolixus.*

Introduction

In many insects, the attainment of a particular size acts as a signal to the neuroendocrine axis that development can proceed to the next stage. In *Manduca sexta*, for example, the endocrine events leading to pupation are delayed until the last stage larva reaches a mass of 5 g (Nijhout and Williams, 1974). Perhaps the best known example is the blood-feeding hemipteran *Rhodnius prolixus*, in which the ingestion of a sufficient blood meal is required for the release of the developmental hormones governing molting and metamorphosis (Wigglesworth, 1934).

In the adult female of *R. prolixus*, the size of the blood meal determines the number of eggs produced (Ruegg and Davey, 1979). Our analysis has led to the conclusion that the female monitors the amount of blood remaining in the mesenteron, where it is stored. When the reserves fall below a particular level, egg production ceases as a consequence of the inhibition by the brain of the production of juvenile hormone by the corpus allatum (Davey *et al.* 1986). These facts imply that the insect is able to detect the distension or weight of the mesenteron, and we have identified non-adapting pressure-sensitive sense organs that are well placed to carry out that task (Chiang and Davey, 1988; Chiang *et al.* 1990).

It has been an implicit assumption of most work that has connected the size or mass of an insect to developmental events that the information detected peripherally would be transmitted to the neuroendocrine axis by nerves (see reviews by Edwards, 1967; Williams, 1980), although this assumption has seldom been subject to rigorous experimental testing. For egg production in *R. prolixus*, we have demonstrated that severing the dorsal vessel sharply reduces egg production because, in such animals, inhibition of the corpus allatum by the brain is not lifted by feeding (Davey and Chiang, 1989; Chiang and Davey, 1990). Experiments with dyes and other observations have demonstrated that the pattern of blood flow is from the abdomen to the brain. Severing the aorta prevents or severely limits the forward transport of hemolymph from the abdomen to the brain, while the diffusion of material from the brain to the abdomen is not unduly restricted (Chiang and Davey, 1990). Conversely, severing the ventral nerve cord anterior to the prothoracic ganglion does not disturb the relationship between the weight of the mesenteron and egg production (Davey, 1993). These observations and other considerations have suggested strongly that severing the dorsal vessel prevents the transport forward of a humoral factor that is released in the abdomen and which activates the

*Author for correspondence.

neuroendocrine axis controlling egg production (Chiang and Davey, 1990). Thus far, however, we have been unable to test this hypothesis in a direct way.

Such an experimental test is possible using the release of the ovulation hormone (myotropin). This myotropic peptide originates in 10 large and easily identified neurosecretory cells in the pars intercerebralis of the brain (Davey, 1967; Sevala *et al.* 1992). It is released on two occasions during each gonotrophic cycle. In both mated and virgin females, it is released immediately after the feed that initiates the cycle and, in mated females, it is again released when mature eggs are present in the ovary (Davey and Kriger, 1985).

We have previously identified and measured the electrophysiological correlates of release in these cells during the second release. In brief, in mated females on the sixth day after feeding (when the myotropin is being released), the cells exhibit a marked increase in frequency of electrical discharges. Moreover, these action potentials are organised as bursts in a phasic pattern. In similar virgin females, which do not release myotropin, the spike frequency is lower. In addition, factors that are known to cause the release of the hormone, such as ecdysone, also cause an increase in spike frquency in these cells (Ruegg *et al.* 1982). Because the myotropin cells are large and easily identified, because the electrophysiological correlates of release are known and because the cells also release the myotropin after feeding, this system presents an opportunity to test this hypothesis in a direct way. The present paper therefore explores the effect of severing the dorsal vessel on the release of myotropin, as detected by electrophysiological events, and on the capacity of hemolymph and tissue extracts to induce the electrophysiological correlates of release in the cells.

Materials and methods

Rhodnius prolixus Stål (Hemiptera: Reduviidae) were reared on rabbit blood and maintained at 28 °C in humid incubators. Adult females, 3–6 days post-emergence, were mated prior to experiments, and females were fed 10–14 days after emergence.

In order to sever the dorsal vessel, females were immobilized with modelling clay, and a small flap was cut, using a scalpel fashioned from a piece of razor blade, in the dorsal cuticle over the dorsal vessel in abdominal segment III. The exposed dorsal vessel was cut with iridectomy scissors, the flap of cuticle was replaced over the wound, and the wound was sealed with Tackiwax (Cenco, Toronto, Canada). Controls consisted of animals in which the dorsal vessel was exposed, but not severed, and the cuticular flap sealed with Tackiwax. Another group of controls consisted of unoperated animals. Surgery was performed on day 3 or 4 post-emergence, and all operated animals were allowed to recover for 7 days before feeding or further experimental manipulation.

For electrophysiological study, an animal was mounted in modelling clay, on the surface of an inverted glass dish, with the head area exposed. A saline-filled chamber was constructed with a small ring of modelling clay around the head. For recording from the large median neurosecretory cells (MNSCs), a small piece of cuticle over the appropriate area was removed with a scalpel. The chamber was filled with *Rhodnius* saline (Maddrell and Gee, 1974). The neural sheath overlying the MNSCs was carefully disrupted with a heat-sharpened glass electrode.

Recording of MNSC activity was accomplished by applying a Ringer-filled extracellular suction electrode (internal diameter 7 μm) to one of the MNSC cells known to be the source of the ovulation hormone. Care was taken to contact only one of the cells, which are large and lie near the surface of the brain, and to ensure that the activity detected was from the contacted cell. Signals were recorded with respect to an indifferent electrode placed in the pool of saline or hemolymph surrounding the head, amplified by a DAM-5A differential preamplifier (W-P Instruments, Inc., Sarasota, FL, USA), viewed on a 5113 oscilloscope (Tektronix, Inc., Beaverton, Oregon) and stored in digital format on video tape using a digital audio processor (Technics, Mississauga, Ontario, Canada) and a video cassette recorder. This procedure has been shown to result in no loss of data (Chiang *et al.* 1985). Spike frequency was used as a measure of cell activity, since an increase in this variable has been shown to be associated with release of the myotropin (Ruegg *et al.* 1982). The frequency of discharge, measured as spikes per 1 s interval, was determined using a model 121 window discriminator (W-P Instruments, Inc.), with the window adjusted manually so as to capture all of the spikes in a particular recording, and a rate/interval analyzer (Frederick Haer & Co., Brunswick, Maine). For each determination of spike frequency, a continuous record of at least 10 min duration was analysed, and the median spike frequency determined. In the figures, the time after feeding is the time at which the animals were prepared for recording. In most cases, recordings were completed within 1 h. Preparations remained viable for considerably longer.

For studies involving the effects of hemolymph or tissue extracts on the electrophysiological activity of MNSCs, the saline surrounding the head was replaced by the material to be tested, and the cell activity was recorded as described above. Crude tissue extracts were prepared by homogenizing the tissue in Ringer and centrifuging at 5000 g for 10 min at 25 °C. The supernatant was used in the electrophysiological assay at an approximate concentration of 1 tissue-equivalent per 100 μl. Hemolymph was collected in a micropipette from the cut end of one leg. The saline covering the brain was replaced by hemolymph. Heat treatment of hemolymph consisted of incubation in a waterbath at 90 °C for 5 min, followed by centrifugation at 5000 g for 5 min at 25 °C to remove the protein clot. The effects of proteases were determined by adding 100 μl of hemolymph to 100 μl of either pronase (70 000 PUK g^{-1}, Calbiochem, San Diego, USA) or trypsin (twice crystallised, 196 i.u. mg^{-1} protein, Freehold, NJ, USA) in saline solution at 1 mg ml^{-1} and incubating at 28 °C for 30 min, followed by heat denaturation of the enzyme at 90 °C for 5 min. Samples were then used in the electrophysiological

assay. Control preparations consisted of diluting and incubating the hemolymph in solutions lacking the enzymes. These control preparations retained significant activity.

Where statistical tests were deemed to be necessary, a Student's *t*-test was employed on pairs of determinations. In most cases, however, differences in spike frequency resulting from different treatments were very large and were not tested for differences.

To determine the effect of severing the aorta on egg laying after feeding, 10 fed, mated females with the dorsal vessel severed were compared with 10 sham-operated females. Two days after feeding, the number of eggs laid by each female was noted, and the females were dissected to determine the number of mature eggs remaining in the ovaries and oviducts.

Results

The electrical activity of the MNSCs was characterized by spontaneously occurring action potentials. In those preparations that were most active, the activity consisted of bursts of phasic activity, as illustrated by Ruegg *et al.* (1982), and resembled most closely the type A action potentials recorded from release sites in the aorta (Chiang *et al.* 1989). There was no evidence of more than one type of action potential, and all cells sampled appeared to behave in a similar fashion. There was no significant difference in the spike frequency of sham-operated and unoperated controls; the data for these two groups were therefore combined. For normal or sham-operated insects, the spike frequency rose within 5 min of the cessation of feeding relative to that of unfed insects. The spike frequency of unfed insects was always less than 4 Hz (N=86).

Effect of severing the dorsal vessel on MNSC activity

Fig. 1 shows the spike frequency, recorded at various times after the cessation of feeding, from intact or sham-operated females and from females with their dorsal vessel severed. There was considerable variation in the spike frequency among individual females. Nevertheless, the spike frequency observed in females with the dorsal vessel severed was always lower than that in control females. The difference in the means at 4 h post-feeding is not statistically significant. The physiological significance, if any, of the transitory increase in spike frequency in operated females at this time is not clear. Even at 2 days after feeding, the MNSC spike frequency of animals with their dorsal vessel severed was significantly lower than that of their control counterparts.

Effect of hemolymph and tissue extracts on MNSC activity

The effect on the MNSC spike frequency of applying hemolymph from various sources directly to the brain of animals with their dorsal vessel severed is shown in Fig. 2. Saline, or hemolymph taken from unfed females, had no effect on the spike frequency, while hemolymph from normal animals at 1, 3, 4.5 and 6 h post-feeding markedly increased the MNSC spike frequency of fed females and of those with

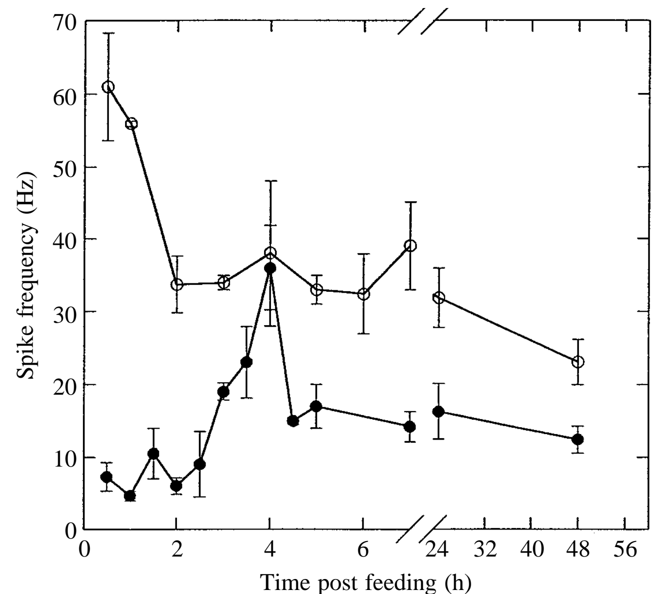

Fig. 1. The median neurosecretory cell (MNSC) spike frequency (Hz) of adult female *Rhodnius prolixus* with their dorsal vessel severed (filled circles) and in controls (open circles). Each point represents the mean from 10–15 animals, with vertical bars showing the standard error of the mean. Each time interval is the mean for a separate group of animals.

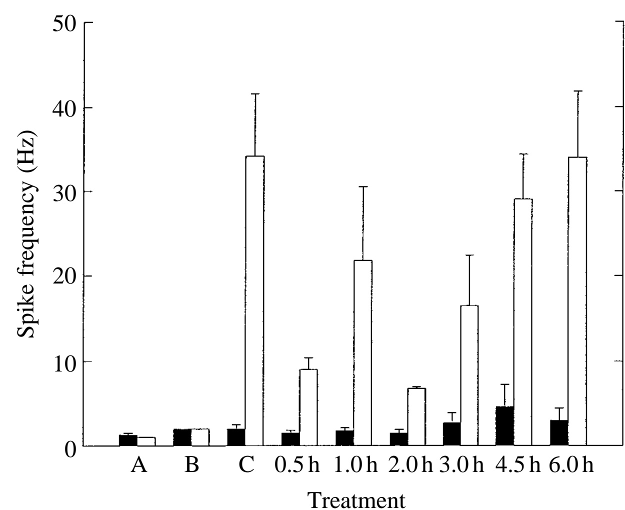

Fig. 2. The effect of hemolymph on the MNSC spike frequency (Hz) of adult female *Rhodnius prolixus* with their dorsal vessel severed before feeding. Recordings were taken from the test animals prepared at 2 h post-feeding and were completed within 2 h. Filled bars (on the left) show spike frequency before application of test material, and open bars (on the right) show spike frequency after application of test material. (A) Saline control; (B) hemolymph from unfed animal; (C) hemolymph from a fed animal with dorsal vessel severed; and the numbers 0.5–6 indicate hemolymph taken from normal animals at the indicated time (in h) post-feeding. Each value is the mean value of four or five females, and the vertical lines indicate the standard error of the mean.

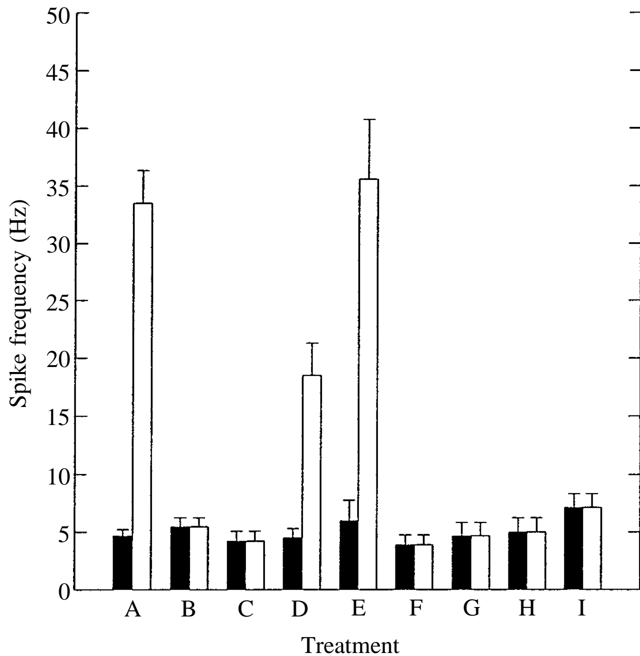

Fig. 3. The effects of various extracts on the MNSC spike frequency (Hz) of adult female *Rhodnius prolixus* with their dorsal vessel severed before feeding. Recordings were taken from animals prepared 2 h after feeding and were completed within 2 h. Filled bars (on the left) show spike frequency before application of test material and open bars (on the right) show spike frequency after application of test material. (A) Heat-treated hemolymph; (B), trypsin-treated hemolymph; (C) pronase-treated hemolymph; (D) heat-treated hemolymph incubated as for the proteases, but without enzyme present; (E) crude extract of thoracico-abdominal ganglion; (F) crude extract of abdominal neurosecretory organ; (G) crude extract of mesenteron; (H) crude extract of midgut; (I) crude extract of hindgut. Values represent the mean of 10–15 animals, and the vertical lines indicate the standard error of the mean.

the dorsal vessel severed. Moreover, hemolymph from a female with a severed dorsal vessel, 6 h post-feeding, also restored the spike frequency in experimental animals, suggesting that severance of the dorsal vessel does not affect the release of the hemolymph-borne factor(s) responsible for increasing the MNSC activity in intact females. These data confirm that the lower spike frequency in females with the dorsal vessel severed is the result of the absence from the hemolymph bathing the brain of a factor present in the hemolymph of normal females and those with the dorsal vessel severed. The data support the view that severing the aorta prevents a factor, or factors, present in the hemolymph in the abdomen from reaching the brain.

Fig. 3 provides information about the characteristics and possible origin of the factors(s) in the hemolymph. The activity in the hemolymph is stable to heating at 90 °C, but sensitive to digestion by trypsin and pronase. Crude extracts of the fused ganglionic mass in the thorax contained significant activity. In contrast, extracts of various parts of the digestive tract, or of the abdominal neurosecretory organs situated outside the central nervous system (Davey and Kuster, 1981), had no effect on the spike frequency of the MNSCs of animals with the dorsal vessel severed. We tentatively conclude that the material in the hemolymph that increases the spike frequency of the MNSCs is very probably a neuropeptide or neuropeptides originating in the fused thoracico-abdominal ganglia in the thorax.

Effect of severance of the dorsal vessel on egg laying

The 10 sham-operated females laid 37 eggs, and dissection of the females revealed only two mature eggs still within the ovary. By contrast, the females in which the dorsal vessel had been severed deposited only four eggs, and a total of 40 mature eggs was found upon dissection.

Discussion

The extracellular suction electrode technique (Chiang *et al.* 1989; Orchard and Steel, 1980; Ruegg *et al.* 1982) employed here allows electrical activity in the large MNSCs of the brain to be monitored *in situ* for extended periods and with minimal surgical manipulation. Since increased spike frequency is a characteristic of neurohormone release from *R. prolixus* neurosecretory cells (Chiang *et al.* 1989; Ruegg *et al.* 1982), the spike frequency of the MNSCs can reasonably be used as a measure of hormone release.

Our results demonstrate that the spike frequency of the MNSCs is significantly depressed after blood feeding in females with the dorsal vessel cut compared with that of their control counterparts. Therefore, there is no doubt that dorsal vessel severance, which severely impedes the forward movement of hemolymph into the head (Chiang and Davey, 1990), diminishes the neurosecretory activity of the large cerebral MNSCs which produce and secrete the ovulation hormone known to be released after feeding (Davey and Kriger, 1985). Moreover, the bout of oviposition that normally follows feeding in both mated and virgin females was all but abolished in females with the dorsal vessel severed. This observation further supports the conclusion that severing the dorsal vessel prevents the release of the ovulation hormone.

The transitory increase in spike frequency at 4 h post-feeding is strikingly at variance with the other data. An explanation is not obvious. It is unlikely to be a consequence of surgical trauma, since the time between surgery and the recording was several days, and since females with the dorsal vessel severed are capable of nearly normal egg production under the appropriate conditions (Davey and Chiang, 1989). It is known that there are several inputs capable of activating the MNSCs (Ruegg *et al.* 1982), and it is possible that one of the pathways leading to activation is stimulated by some event 4 h after feeding. Since the MNSCs in mated females are already activated, this transitory effect is not manifest in them.

The data presented in this paper also show that the hemolymph of fed females contains one or more factors which restore the spike frequency in the MNSCs in females with the dorsal vessel severed. The factor is absent from the

hemolymph of unfed females and appears to be a neuropeptide originating in the ganglionic mass in the thorax.

It is interesting to note that hemolymph taken several hours post-feeding from a female with a severed dorsal vessel was able to restore the MNSC activity in another similarly treated female. The operation did not interfere with the release of the factor responsible for restoring the MNSC activity to the level in the controls.

Fig. 2 reveals an apparently biphasic titer of activity in the hemolymph of fed females, in which the activity rises and then decreases after the first hour, to rise slowly thereafter. This can be related to two possible causes. First, the taking of a blood meal will result in a sharp increase in hemolymph volume. While this has not been documented for the period immediately after the feed, even at 2 days post-feeding, the volume has increased by a factor of 4 (Kuster and Davey, 1983). This massive dilution is accompanied by a brisk diuresis, which has not been documented for adults. These two related events no doubt combine to produce a very dynamic environment which might dilute and/or remove from the circulation various hormones. Second, stimulation of the MNSCs to release their product is known to stimulate protein synthesis in the cells (Davey and Prasher, 1990), and it is possible that the biphasic appearance of activity in the hemolymph is also partly a result of hormone synthesis catching up to the rate of release of the hormone.

This information can be integrated with what is already known about the inputs governing egg production in *R. prolixus*. Previous work from this laboratory has shown that it is the quantity of blood stored in the mesenteron that is the primary determinant of the number of eggs produced. The insect monitors the blood remaining in the mesenteron and, when it falls below 35 mg in mated females or 48 mg in virgin females, egg production ceases because the brain imposes an inhibition on the corpus allatum (Davey *et al.* 1986). Pressure-sensitive receptors located in ventral abdominal wall appear to be the best candidates for the detection of abdominal distension or weight. The blood-borne factor(s) described in the present paper represent a new link in the chain of events connecting the abdomen to the neuroendocrine axis controlling reproduction. We have used the readily detectable electrophysiological correlates of release of the ovulation hormone as an indicator of the activation of the neuroendocrine axis. This is far from being a simple system, since these cells are known to receive other inputs later in the gonotrophic cycle which no doubt override the input described in this paper. While it would therefore be naive to regard the factor as the only, or even the principal, source of stimulation for the release of the ovulation hormone, these experiments provide direct evidence for the existence of a substance carried to the brain by the dorsal vessel.

These experiments do not, however, directly prove that the factor from the abdomen is the factor postulated in earlier papers to be responsible for lifting the inhibition by the brain of the release of juvenile hormone by the corpus allatum. However, all of the facts currently known are consistent with the following working hypothesis. The information detected by the pressure receptors is conducted to the brain by neuropeptide(s) and, so long as this material is reaching the brain, the inhibition of the corpus allatum is lifted. When the neuropeptide(s) no longer reaches the brain, the inhibition is re-imposed, the corpus allatum ceases to secrete juvenile hormone and egg production halts. The source of the factor(s) appears to be some part(s) of the fused ganglionic chain; information from the sense organs is presumably conducted to the source of the factor by nerves.

The site of release of the factor(s) is not clear. It is possible that it might be released into the hemolymph in the abdomen by thoracic neurosecretory cells with axons in the abdominal nerves. If that is so, it complicates the interpretation of experiments that examine the effects of severing such nerves on egg production (Davey, 1982).

Do these experiments on the control of egg production have anything to contribute to the study of the inputs controlling larval development in insects? It is already clear that severing the dorsal vessel in a fourth-stage larval *R. prolixus* either prevents development entirely, so that the operated insects do not molt, or severely retards development, so that operated insects do not molt until 45 days after feeding rather than after the 14 days normally required in intact insects. For the insects that do moult, larval–adult intermediates are produced. These unpublished observations (R. P. Ruegg and K. G. Davey) recall similar observations made by Wigglesworth (1934), in which severing the dorsal vessel of fifth-stage larvae delayed molting. Because spike frequency in the corpus cardiacum can be used as an index of the release of prothoracicotropic hormone (PTTH) (Orchard and Steel, 1980), we have been examining the effect of dorsal vessel severance on spike frequency in the corpus cardiacum. These experiments are not yet complete, but they indicate that the signal to release PTTH may also reach the brain by a humoral route. However, our unpublished data also show that the diuresis that follows feeding in *R. prolixus* is undiminished in animals with the dorsal vessel severed. Since diuretic hormone is released by feeding (Maddrell, 1964), this suggests that there may be two mechanisms operating. One is humoral in nature and acts on developmental events, while the other travels in nerves and acts more rapidly on the events associated with homeostasis. Diuresis in *R. prolixus* ensues immediately after the initiation of feeding, so that the first drops of urine are passed while the insect is still feeding (Maddrell, 1964).

A recent paper (Yin *et al.* 1994) has implicated a peptide from the gut in the stimulation of the neuroendocrine axis leading to egg development in the blowfly *Phormia regina* using an assay that measures increased egg production in females exposed to a combination of endogenous hormone and injected hormone from donor flies. The relationship between this gut peptide and the factor described here is not clear.

Finally, it should be noted that Williams (1977) proposed that the attainment of a mass of 5 g by larvae of *Manduca sexta* caused the release of a factor in the abdomen that acted on the

brain to initiate the endocrine events leading to metamorphosis. Unfortunately, the data supporting this provocative assertion have never been published.

We are grateful to Dr Gary Chiang for helpful discussions during the course of this study. Research in this laboratory is supported by the Natural Sciences and Engineering Research Council of Canada.

References

Chiang, R. G., Chiang, J. A. and Davey, K. G. (1989). Electrophysiology and ultrastructure of axons of neurosecretory cells associated with the cephalic aorta of *Rhodnius prolixus*. *J. Insect Physiol.* **35**, 209–222.

Chiang, R. G., Chiang, J. A. and Davey, K. G. (1990). Structure of the abdominal receptor responsive to internally applied pressure in the blood-feeding insect, *Rhodnius prolixus*. *Cell Tissue Res.* **261**, 583–587.

Chiang, R. G. and Davey, K. G. (1988). A novel receptor capable of measuring applied pressure in the abdomen of an insect. *Science* **241**, 1665–1667.

Chiang, R. G. and Davey, K. G. (1990). Circulation is required for egg production in the insect *Rhodnius prolixus*. *Invert. Reprod. Dev.* **18**, 177–183.

Chiang, R. G., Knobloch, C. A., Singleton, D. M., Steel, C. G. H. and Davey, K. G. (1985). Recording electrophysiological data on videotape: a superior and less costly alternative to conventional tape recorders. *J. Neurosci. Meth.* **15**, 15–20.

Davey, K. G. (1967). Some consequences of copulation in *Rhodnius prolixus*. *J. Insect Physiol.* **13**, 1629–1636.

Davey, K. G. (1982). The effect of severing abdominal nerves on egg production in *Rhodnius prolixus*. *J. Insect Physiol.* **28**, 509–512.

Davey, K. G. (1993). How is information about meal size transmitted to the endocrine system in *Rhodnius*? In *Host Regulated Developmental Mechanisms in Vector Arthropods* (ed. D. Borovsky and A. Spielman), pp. 3–10. Vero Beach, FL, USA: University of Florida.

Davey, K. G. and Chiang, R. G. (1989). The effect of severing the dorsal vessel on egg production in *Rhodnius prolixus*. *Archs Insect Biochem. Physiol.* **11**, 139–146.

Davey, K. G. and Kriger, F. L. (1985). Variations during the gonotrophic cycle in the titre of the myotropic ovulation hormone and the response of the ovarian muscles in *Rhodnius prolixus*. *Gen. comp. Endocr.* **58**, 452–457.

Davey, K. G. and Kuster, J. E. (1981). The source of an antigonadotropin in the female of *Rhodnius prolixus*. *Can. J. Zool.* **59**, 761–764.

Davey, K. G., Maimets, I.-K. and Ruegg, R. P. (1986). The relationship between crop size and egg production in *Rhodnius prolixus*. *Can. J. Zool.* **64**, 2654–2657.

Davey, K. G. and Prasher, A. K. (1990). Increased protein synthesis is closely coupled to release in the myotropic neurosecretory cells of *Rhodnius prolixus*. *Insect Biochem.* **20**, 215–220.

Edwards, J. S. (1967). Neural control of development. In *The Invertebrate Nervous Systems, Their Significance for Mammalian Physiology* (ed. C. A. G. Wiersma), pp. 95–111. Chicago: Chicago University Press.

Kuster, J. E. and Davey, K. G. (1983). The effect of allatectomy or neurosecretory cell ablation on protein synthesis in the spermathecae of *Rhodnius prolixus*. *Int. J. Invert. Reproduct.* **6**, 189–195.

Maddrell, S. H. P. (1964). Excretion in the blood-sucking bug, *Rhodnius prolixus* Stål. III. The control of the release of the diuretic hormone. *J. exp. Biol.* **41**, 459–472.

Maddrell, S. H. P. and Gee, J. G. (1974). Potassium-induced release of the diuretic hormone of *Rhodnius prolixus* and *Glossina austeni*: Ca^{2+} dependence, time course and localization of neurohaemal areas. *J. exp. Biol.* **61**, 155–171.

Nijhout, H. F. and Williams, C. M. (1974). Control of molting and metamorphosis in the tobacco hormworm, *Manduca sexta* (L): growth of the last-instar larva and the decision to pupate. *J. exp. Biol.* **61**, 481–491.

Orchard, I. and Steel, C. G. H. (1980). Electrical activity of neurosecretory axons from the brain of *Rhodnius prolixus*: relation of changes in the pattern of activity to endocrine events during the molting cycle. *Brain Res.* **191**, 53–65.

Ruegg, R. P. and Davey, K. G. (1979). The effect of C18 juvenile hormone and Altosid on the efficiency of egg production in *Rhodnius prolixus*. *Int. J. Invert. Reprod.* **1**, 3–8.

Ruegg, R. P., Orchard, I. and Davey, K. G. (1982). 20-Hydroxy-ecdysone as a modulator of electrical activity in neurosecretory cells of *Rhodnius prolixus*. *J. Insect Physiol.* **28**, 243–248.

Sevala, V. L., Sevala, V. M., Davey, K. G. and Loughton, B. G. (1992). A FMRFamide-like peptide is associated with the myotropic ovulation hormone in *Rhodnius prolixus*. *Archs Insect Biochem. Physiol.* **20**, 193–203.

Wigglesworth, V. B. (1934). The physiology of ecdysis in *Rhodnius prolixus* (Hemiptera). II. Factors controlling molting and 'metamorphosis'. *Q. Jl microsc. Sci.* **77**, 191–222.

Williams, C. M. (1977). Anti-juvenile hormone effects of a diffusible agent that inhibits the corpora allata. *Pont. acad. sci. scripta varia.* **41**, 1–8.

Williams, C. M. (1980). Growth in insects. In *Insect Biology in the Future* (ed. M. Locke and D. S. Smith), pp. 369–383. New York, London: Academic Press.

Yin, C.-M., Zou, B.-X., Li, M.-F. and Stoffalano, J. G. (1994). Discovery of a mid-gut peptide hormone which activates the endocrine cascade leading to öogenesis in *Phormia regina* (Meigen). *J. Insect Physiol.* **40**, 283–292.