J. Exp. Biol. (1972), 57, 755–763
With 1 *text-figure*
Printed in Great Britain

THE CONTROL OF CHANGES IN PERIPHERAL SENSILLA ASSOCIATED WITH FEEDING IN *LOCUSTA MIGRATORIA* (L.)

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(*Received* 1 *May* 1972)

INTRODUCTION

It has been shown that changes in the electrical resistance across the tips of the maxillary palps of *Locusta migratoria* (L.) occur in relation to feeding (Bernays, Blaney & Chapman, 1972). These changes in resistance reflect changes in the individual chemoreceptors on the dome, such that immediately after a meal the resistance is high and the sensilla do not respond to stimulation, whereas about 2 h after feeding resistance is low and the sensilla do respond. In this paper the mechanisms effecting the relationship between feeding and the changes in palp sensilla are investigated.

The basis of the experimental method was the measurement of resistance across the tips of the palps after subjecting the insects to various treatments. In general the insects were deprived of food for 3·5–4·5 h so that the foregut was almost empty (Bernays & Chapman, 1972) and then the effects of feeding were simulated by filling the foregut with agar. The resistance was measured 5–10 min later.

The pathways involved were investigated by cutting various nerves associated with the foregut and by injecting haemolymph or corpus cardiacum homogenates. Operations on the nervous system were performed 24 h before the crop was filled with agar and resistance across the palps was measured.

MATERIALS AND METHODS

Insects

All the experiments were carried out on male fifth-instar larvae of *Locusta migratoria*. These were taken from the normal laboratory stock at the Centre for Overseas Pest Research on the day of moulting, and subsequently maintained in 12 l cylindrical cages containing not more than 50 insects each. The locusts were fed daily on grass, and maintained at 30–35 °C during the day, and 28 °C at night. Under these conditions the fifth instar lasted 9 days. Insects were used in experiments 3–5 days after ecdysis, and in any one experiment all the insects were of the same age (± 12 h). They were kept in the light at 30 °C in the 4 h prior to testing.

Electrical resistance

The measurement of electrical d.c. resistance across the tips of the maxillary palps is described in detail elsewhere (Bernays *et al.* 1972). In some cases the insects

were anaesthetized with CO_2 before fixing them down in order to make the measurements. This lowered the values of resistance obtained by about 20%, although the same pattern of change in relation to feeding was seen. The experiments employing anaesthesia are clearly indicated in the tables.

Artificial filling of the foregut

The foregut was filled artificially with 3–5% agar solution used just as it was setting. This is an extremely viscous solution but can be pushed into the crop via the oesophagus through a cannula (Portex green, O.D. = 0·63 mm) attached to a syringe. With the right consistency of agar the crop can be distended to the levels found after a meal on a most favoured food, e.g. *Agropyron* (Bernays & Chapman, 1972). Starting with an empty foregut, the volume of a full meal is about 120 μl, and this was the amount of agar used. When the foregut had been filled, agar spilled out of the mouth and this could be detected easily because the labrum was raised to insert the cannula at the base of the mandibles. The agar in the gut set hard, and could not be moved without digestion, a process which took up to 12 h. If the agar in the gut was too liquid it did not distend the crop in the normal manner, but appeared at the mouth when as little as 70 μl had been inserted. It also passed back to the midgut very rapidly and appeared in the faecal pellets within 2 h.

Operations on the stomatogastric system

All the operations on the stomatogastric system were performed without the use of saline baths or irrigation. Instruments were washed in absolute alcohol before each operation, and insects were anaesthetized with CO_2. The different operations performed are shown in Fig. 1, but two different sites of entry were required.

Anterior regions were approached through the frons. First a transverse incision was made between the frontal carinae, just below the median ocellus. From either end of this, vertical cuts were made just inside each frontal carina to the epistomal sulcus. Thus a flap of cuticle was cut out on three sides and could be folded vertically down. Numerous small air sacs and two larger ones were then removed to reveal the frontal ganglion and its associated nerves. The recurrent nerve or the recurrent nerve plus posterior pharyngeal nerves or both frontal connectives (operations 3–5, Fig. 1) were severed. After the operation, the flap of cuticle was returned to its normal position, and no further sealing was required.

The posterior pharyngeal nerves are very variable in position, and since variation could not be investigated in every case insects where the relevant nerve was not found in a short time were rejected. In some cases the posterior pharyngeal nerve unites on one or both sides with the recurrent nerve, and it is probable that on some occasions, in performing operation 3, the recurrent nerve was severed anterior to such a union. Also, it is probable that occasionally the posterior pharyngeal nerve was represented by a number of smaller nerves. Anatomical details are given by Allum (unpublished)

The oesophageal nerves and nervi corpora cardiaca were approached through the cervical membrane dorsally. Insects were fixed down either with the head in plasticine or in a jig similar to that used by Staal (1961). The exact position depended on which nerves were to be severed. The membrane was cut transversely and the median cephalic air sac was removed. The nerves were individually severed on either side.

and at the completion of any operation the head was fixed to the pronotum with a warm beeswax–resin mixture to seal the wound.

All insects were allowed to recover overnight at 26 ± 2 °C with no food present. Out of over 250 operated insects there were only five deaths in this period.

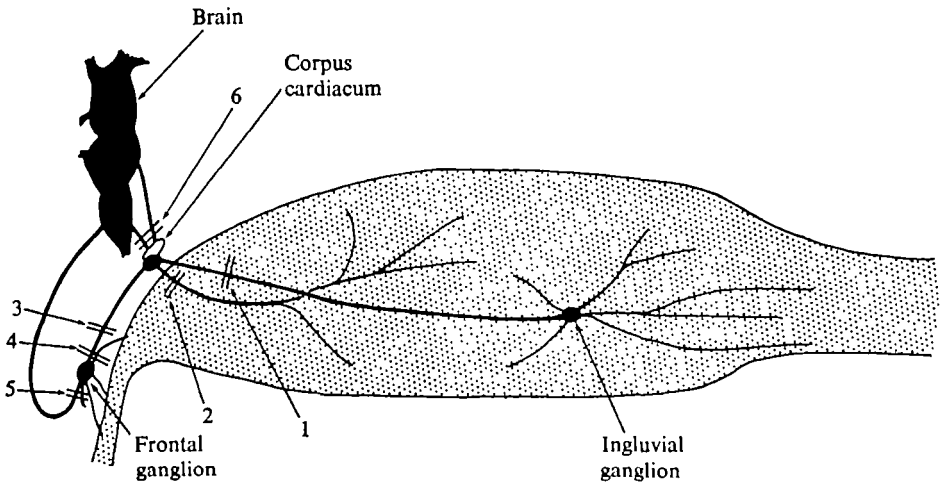

Fig. 1. Diagram to illustrate the positions of different nerve-severing operations. 1, Outer oesophageal nerves cut; 2, inner oesophageal nerves cut; 3, recurrent nerve cut; 4, recurrent nerve and posterior pharyngeal nerves cut; 5, frontal connectives cut; 6, nervi corpora cardiaca cut.

Injections

Larvae were injected immediately before testing using no. 20 'Record' hypodermic needles and an 'Agla' micrometer syringe. Material was injected between abdominal tergites 4 and 5 just above the left spiracle. Between 4·5 and 6 min were allowed to elapse between the time of injection and the measurement of resistance. Preliminary experiments in which 100 μl of Amaranth was injected in the same manner indicated that this reached the tips of the palps in 4 min.

For transfusion experiments, samples of haemolymph were obtained by cutting the mesocoxal membrane of donor larvae and drawing up the drop which oozed out under gentle pressure into a syringe half-filled with liquid paraffin. To obtain the standard 100 μl, three donors were required. The haemolymph was injected into test insects immediately after collection.

Corpora cardiaca were removed from 2-day-old fifth-instar larvae and homogenized with a volume of Hoyle's (1953) insect Ringer solution equivalent to 100 μl/pair of glands. The homogenate was stored overnight at 4 °C and each recipient was injected with 100 μl of the homogenate after it had warmed to room temperature. Homogenized fat body was used in control injected insects.

Storage lobes of corpora cardiaca were removed from 5-day-old fifth-instar larvae and homogenized with Ringer to give a solution equivalent to 50 μl/pair of lobes. The homogenate was stored for 1 week at 4 °C and each recipient was injected with 20 μl (= 0·4 storage lobes), after warming it to room temperature. Hoyle's insect Ringer solution was used for the control injections.

Diuresis

Diuresis changes were monitored by measuring the water content of the faeces produced in the 30 min following distension of the crop with agar. Each insect was kept, after treatment, in a 1 lb jam jar at 30 °C, and faecal pellets were removed with forceps as soon as they appeared and were placed in 2 ml airtight phials. At the end of the test period the faeces were weighed, dried at 100 °C for 2 h and weighed again.

RESULTS

Resistance measurements

(1) *Effect of input from peripheral sensilla*

An attempt was made to discover whether palpation movements, chemical stimulation of the palps, or both together, would themselves cause an increase in resistance from the base-line values found after approximately 4 h of food deprivation.

Table 1. *The effects of different stimuli on the electrical resistance of the tip of the maxillary palp of insects deprived of food for 4 h*

(Values after CO_2 anaesthesia.)

	Experiment	Resistance (KΩ) (mean ±S.E.)	No. of insects
(*a*)	Fed control	94±2·2	10
	Control	70±2·4	10
(*b*)	Palps mechanically stimulated	70±3·4	11
	Control	75±3·0	14
(*c*)	Palps chemically stimulated	69±4·3	9
	Control	71±4·7	9
(*d*)	Palps grass stimulated	78±3·6	16
	Control	74±2·5	16
(*e*)	Handled	70±3·8	7
	Control	72±3·1	7

The importance of the mechanical aspects of palpation was investigated by making the insects walk on the edge of a vertical tin disc which was continuously rotated. The insect was held by the hind femora, and in most cases it walked steadily with the first and second pairs of legs, regularly palpating on the edge of the disc and occasionally biting at it. This was maintained for 5–10 min. Immediately after this activity the electrical resistance across the palp tips was measured. A similar number of undisturbed insects were used as controls. There was no difference between the two groups in the resistance across the palps (Table 1*b*).

To test the effect of chemical stimulation, the insect was fixed in position in the block used for measuring the resistance (Bernays *et al.* 1972), and then whole-grass extract from *Poa* sp. was dripped on to the mouthparts from a plastic cannula for 10 min. Mouthpart movement occurred for much of this time and small quantities were swallowed, but not sufficient to distend the foregut. Again, there was no difference between these insects and control, unstimulated insects (Table 1*c*).

Other insects were made to palpate grass blades (*Poa* sp.) without being allowed to bite them. More or less consecutive palpating could only be obtained for about

min in this way and only those insects were tested which had palpated for over 5 min. Table 1*d* shows that, here too, there was no difference from the control.

As a further control for the above experiments, a group of insects was handled and held by the hind femora, but without any stimuli applied to the mouthparts. The resistance was again measured and compared with untouched insects. There was no indication of a significant change in resistance (Table 1*e*).

(2) *Effect of foregut distension*

Resistance across the palps was measured in insects which had been deprived of food for 4 h at 30 °C and then had their foreguts distended with agar. The resistance was high (90 ± 1·4 KΩ) compared with control insects which did not have the crops filled (70 ± 0·7 KΩ). The change occurred in less than 10 min from the time of agar-filling, well before agar could be recovered from the midgut. Half-filling the foregut with agar did not significantly increase the resistance (Table 2).

Table 2. *The effects of filling the crop with agar on the resistance across the tip of the maxillary palp after various operations*

(Values after CO_2 anaesthesia.)

Operation	Condition of crop	No. of insects	No. of results at different levels of resistance (KΩ) 50–69	70–89	90–109	110–129	Resistance (mean ± S.E.)
Unoperated insects	Full	17	2	6	7	2	90 ± 1·4
	Empty	20	8	10	1	1	70 ± 0·7
Unoperated insects	Half-full	13	5	5	2	1	73 ± 1·7
	Empty	14	6	6	2	—	71 ± 1·5
Outer oesophageal nerves cut	Full	16	4	6	3	3	91 ± 1·4
	Empty	15	8	3	4	—	76 ± 1·0
Recurrent nerve cut	Full	25	6	9	2	8	85 ± 1·9
	Empty	20	4	16	—	—	74 ± 0·6
Recurrent + posterior pharyngeal nerves cut	Full	23	10	8	4	1	78 ± 1·6
	Empty	20	6	10	2	2	76 ± 1·1
Frontal connectives cut	Full	11	2	5	4	—	77 ± 0·7
	Empty	12	1	6	5	—	77 ± 0·8
NCC 1 and 2 cut	Full	18	5	8	5	—	84 ± 4·0
	Empty	18	4	7	6	1	81 ± 3·5

(3) *Effect of operations*

Severing the outer oesophageal nerves had no effect on the normal resistance increase with crop filling (Table 2). After cutting the recurrent nerve, the average increase in resistance following crop filling was less than normal, though the results (Table 2) indicate a bimodality, the resistance in some insects reaching the value for fully fed insects, while in others it remained at the empty level. Severing the recurrent nerve plus the posterior pharyngeal nerves, or the frontal connectives, completely prevented the normal increase in resistance, while cutting the nervi corpora cardiaca (NCC) 1 and 2 together had a similar effect, but in this experiment the mean resistance in empty insects was unusually high (81 ± 3·5 KΩ) with a high level of variation.

(4) *Injections*

Haemolymph was taken from insects 5–10 min after feeding and injected into others which had been deprived of food for 4 h. The resultant resistance across the tips of the palps was as high as that found after feeding. Control insects injected with blood from unfed insects, or with insect Ringer, showed no increase compared with the levels found in unfed control insects (Table 3).

Table 3. *The effects of haemolymph injections on the resistance across the tip of the maxillary palp of insects deprived of food for 4 h*

Treatment	Resistance (KΩ) (mean ± S.E.)	No. of insects
Fed control	114 ± 2·4	15
Untouched control	91 ± 1·5	32
Ringer injected	87 ± 1·7	11
Haemolymph of food-deprived insects injected	90 ± 2·3	13
Haemolymph of fed insects injected	121 ± 3·1	17

Table 4. *The effects of injecting homogenates of corpora cardiaca, or storage lobes of corpora cardiaca, on the resistance across the tip of the maxillary palp of insects deprived of food for 4 h*

Treatment	Resistance (KΩ) (mean ± S.E.)	No. of insects
Control (untreated)	96 ± 2·9	17
Control (fat body homogenate injected)	97 ± 3·4	18
Corpus cardiacum homogenate injected	128 ± 3·4	20
Control (Ringer injected)	97 ± 1·6	10
Corpus cardiacum storage lobe homogenate injected	122 ± 2·1	10

Homogenates of corpora cardiaca taken from donors deprived of food for 4 h were injected into similarly deprived insects. Other insects were injected with homogenized fat body as controls. In a similar experiment, homogenates of the storage lobes of corpora cardiaca were injected, using Hoyle's insect Ringer for the controls. Injection of fat body or saline had no effect on the palp-tip resistance, but injection of either of the homogenates of corpora cardiaca raised the resistance to the level found in recently fed insects (Table 4).

Diuresis

The water content of the faeces of insects was determined following the filling of the foreguts with agar. Unoperated insects excreted significantly more water after filling the foregut than control insects which were handled without filling (Table 5). The faecal water content after agar-filling of insects which had the inner or outer oesophageal nerves cut was also higher than that of control, unfilled, insects, while after severance of the recurrent nerve only a small increase was observed. Filling the

crop with agar did not result in any increase in the water content of the faeces after cutting the recurrent nerve together with the posterior pharyngeal nerves, or after cutting the frontal connectives.

Table 5. *The effect on faecal water content of filling the foregut with agar*

(Insects were deprived of food overnight, and had different nerves of the stomatogastric system severed. Water content is expressed as a percentage of that in the faeces of control insects which had undergone similar operations but which received no agar.)

Treatment	Water content (as % of control values) (mean ± S.E.)	No. of insects
Unoperated	148 ± 6·2	11
Outer oesophageal nerves cut	129 ± 2·0	6
Inner oesophageal nerves cut	120 ± 1·4	9
Recurrent nerve cut	107 ± 3·0	34
Recurrent + posterior pharyngeal nerves cut	98 ± 1·8	9
Frontal connectives cut	96 ± 2·9	12

DISCUSSION

The evidence presented by Bernays *et al.* (1972) shows that changes in the resistance across the tips of the maxillary palps result from changes in the chemoreceptor sensilla. These changes are associated with feeding and could arise in one of two ways: the act of feeding might have some direct effect on the sensilla such that their terminal openings were occluded, or occlusion might be effected via some internal controlling mechanism.

Stimulation of the sensilla of the palps, either mechanically or chemically, without permitting feeding, did not produce changes in resistance. It therefore seems clear that there was no direct effect of feeding on the sensilla and that the changes observed were brought about by some internal mechanism. This was confirmed by the experiments in which the crop was distended with agar. The increase in resistance which followed suggests that there is some link between the crop and the sensilla.

Plotnikova (1967) depicts large numbers of stretch receptors on the posterior parts of the foregut of *Locusta*, while Clarke & Langley (1963) describe six pairs of probable stretch receptors in the pharyngeal region. Half-filling the foregut does not affect palp resistance, but expanding it with a volume of agar equivalent to that of a full meal causes resistance to rise to the same levels as after feeding. It thus appears that the expansion of the foregut, presumably monitored by stretch receptors, initiates the sequence of events leading to the increase in palp resistance.

The results of operations on the stomatogastric system show that information about stretching of the foregut is fed to the frontal ganglion via the posterior pharyngeal nerves. This implies that it is only the anterior region of the foregut, where Clarke & Langley (1963) observed stretch receptors, that is important in this respect. It is significant that this is the last part of the foregut to become distended in taking a full meal (Bernays & Chapman, unpublished). The arrangement of the nerves is variable (Allum, unpublished) and cutting the recurrent nerve sometimes also involves cutting one or both of the posterior pharyngeal nerves. Where the posterior pharyngeal

nerves are separate from the recurrent nerve, cutting the recurrent nerve has no effect on the normal increase in resistance after filling the foregut. Where the posterior pharyngeal nerves branch from the recurrent nerve, the operation can prevent the normal increase.

The posterior pharyngeal nerves run to the frontal ganglion, and the surgical experiments indicate that from here information on anterior foregut distension is passed to the brain via the frontal connectives. If these are cut, no increase in resistance follows gut distension. The subsequent nerve pathways have not been elucidated.

The experiments with haemolymph from fed and unfed animals and with homogenates of corpora cardiaca indicate that a hormone is released from the storage lobes of the corpora cardiaca and that this, circulating in the haemolymph, provides the final link with the sensilla. The nervi corpora cardiaca presumably provide the link between the brain and corpora cardiaca so that cutting these nerves would be expected to prevent hormone release from the corpora cardiaca and the consequent rise in resistance. The unusually high levels obtained in control insects following this operation might be accounted for in terms of leakage of neurosecretory material from the cut ends of the nerves since Highnam & West (1971) have shown that the convolutions of the neurosecretory axons of these nerves in the pars intercerebralis form an extensive reservoir within the brain.

The results of the experiments on diuresis indicate that anterior foregut distension and the same nerve pathway are involved in diuretic hormone release. It is well known that feeding causes diuresis (e.g. Norris, 1961; Highnam, Hill & Mordue, 1966), and that diuretic hormone is a neurohormone stored in the storage lobes of the corpora cardiaca (e.g. Mordue, 1969, 1971; Mordue & Goldsworthy, 1969). Thus both diuresis and changes in the palp resistance are regulated by feeding in the same way and it is possible, though not proved, that a single hormone may be responsible for both phenomena.

A marked increase in palp resistance was observed within 10 min of feeding, and the water content of the faeces rose by over 40% in the 30 min following a feed, suggesting that the hormone released from the corpora cardiaca was acting very rapidly. Mordue (1969), however, has shown that in adult *Schistocerca gregaria* the amount of hormone released in such a short time is likely to be very small. He has further shown that small amounts of corpus cardiacum homogenate have very little effect on diuresis. There is thus a discrepancy between his work on *Schistocerca* and this work on *Locusta*, which cannot at present be resolved.

SUMMARY

1. The electrical resistance across the tips of the maxillary palps is not affected by stimulation of the palps, but increases to the normal level found after feeding as a result of distension of the foregut with agar or injection of corpus cardiacum homogenates into the haemolymph.

2. No increase in resistance occurs if the posterior pharyngeal nerves or the frontal connectives are cut.

3. It is inferred that distension of the foregut stimulates stretch receptors which, acting via the posterior pharyngeal nerves, the frontal connectives and the brain,

cause the release of hormone from the storage lobes of the corpora cardiaca. This hormone acts on the terminal sensilla of the palps, causing them to close and so increasing the resistance across the palps.

4. Release of the diuretic hormone is controlled via the same pathway.

We wish to thank Mrs A. J. Mordue for preparing the extracts of corpora cardiaca, and Mr W. M. Blaney, Dr R. C. Cooter, Dr G. J. Goldsworthy, Dr J. E. Moorhouse and Dr W. Mordue for criticizing the manuscript.

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