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MECHANISMS CONTROLLING MODULATION BY HAEMOLYMPH AMINO ACIDS OF GUSTATORY RESPONSIVENESS IN THE LOCUST

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Summary

1. Previous work has shown that fifth-instar nymphs of *Locusta migratoria* (L.) adjust their feeding behaviour to compensate for variation in dietary protein and carbohydrate levels. These changes in behaviour are accompanied by nutrient-specific changes in the responsiveness of taste receptors on the mouthparts.

2. Levels of free amino acids in the haemolymph affect the responsiveness of maxillary gustatory receptors to stimulation by amino acids. The mechanisms mediating this response are investigated.

3. Sectioning the maxillary nerve does not prevent an injection of amino acids into the haemolymph from causing reduced chemo-responsiveness, indicating that centrifugal neural feedbacks are not involved.

4. Isolating the distal two segments of the palp by ligature and then micro-injecting amino acids into the palp tip also causes modulation of responsiveness, showing that the effect is mediated at, or close to, the sensory receptors.

5. Radio-labelling studies indicate that amino acids injected into the abdomen are found in the haemolymph of the palp within the time necessary to cause a peripheral change in gustatory responsiveness.

6. Possible mechanisms enabling amino acids in the blood of the palp to influence sensory responsiveness are discussed. The simplest mechanism consists of amino acids in the haemolymph reaching the sensillum liquor and adapting the receptors to further stimulation from the food.

Introduction

In recent years it has been demonstrated that at least some insects are able to regulate independently their intake of protein and carbohydrate, both by adjusting the amount of a given food that they ingest and by selecting between alternative foods (reviewed by Simpson and Simpson, 1990; Waldbauer and Friedman, 1991).

Fifth-instar nymphs of *Locusta migratoria* eat more of an artificial diet

* Please note that the authors are not related.

Key words: maxillary palp, taste, chemoreceptor, locust, *Locusta migratoria*, feeding.

containing reduced levels of protein than of a higher protein diet and will select a food containing protein after taking only one meal of a food lacking it (Simpson and Abisgold, 1985; Simpson *et al.* 1990*c*). Abisgold and Simpson (1987) found that feeding locusts on artificial diets containing different levels of protein leads to variation in osmolality and in levels of free amino acids in the haemolymph. Composition of the haemolymph, in turn, determines the average time between meals.

We then went on to show that amino acids in the haemolymph modulate the responsiveness of taste receptors on the mouthparts. Such changes could well contribute to variation in intermeal intervals by altering the probability that a locust, on making contact with a food, will commence and maintain ingestion of it (Abisgold and Simpson, 1988; Simpson *et al.* 1990*b*). An injection designed to raise the profile of amino acids in the haemolymph of an insect fed on a low-protein diet to that of a locust fed on a high-protein food resulted in a 60–70 % reduction in the responsiveness of taste sensilla on the maxillary palps to stimulation with an amino acid mix, but had no effect on the response of the same sensilla to sucrose. Similar nutrient-specific changes in gustatory responsiveness were found to accompany dietary selection for both protein and carbohydrate in locust nymphs fed diets lacking either of these classes of nutrient, with responsiveness of maxillary sensilla to sugars and amino acids being modulated separately (Simpson *et al.* 1991). Finally, work on adult locusts showed that changes in the relative responsiveness of maxillary palp chemosensilla to stimulation with amino acids and sugar accompanied the varying ratio of protein and carbohydrate selected by insects throughout the somatic growth phase (Simpson *et al.* 1990*a*).

It would seem, then, that modulation of taste sensitivity plays a significant role in the regulation of protein and carbohydrate intake, in the face of both varying dietary composition and changing nutritional needs. Such a mechanism provides a rapid and direct link between the nutritional state of the insect, as indicated by levels of nutrients in the haemolymph, and its feeding behaviour. An important question is how metabolites such as amino acids in the haemolymph cause the change in responsiveness of mouthpart taste receptors. The various possible mechanisms may be summarized as follows. (1) Chemoreceptors located somewhere in the body (perhaps even within the central nervous system, CNS) monitor levels of metabolites in the haemolymph and send their outputs to the CNS, resulting in centrifugal neural modulation of the mouthpart taste receptors, either by synaptic contact between the efferent neurones and the taste cells or by release of neuroactive compounds in the vicinity of the sensory cells. (2) As above, with the peripheral modulation being caused not by neural efferents but by the release of neuromodulators/neurohormones from the CNS or its associated neuroendocrine structures. (3) Levels of metabolites in the haemolymph modulate the chemosensory neurones, without recourse to the CNS, either directly or *via* a local receptor/effector organ.

In the present paper we report the results of three experiments aimed at examining these possible mechanisms.

Materials and methods

Insects and diets

Locusts were reared in the Department of Zoology, University of Oxford. They were taken as they ecdysed to the fifth instar and each was placed in a 17 cm × 12 cm × 6 cm plastic box containing an expanded aluminium perch and ample seedling wheat and bran. The boxes were kept at 30 °C under a 12h:12 h L:D cycle until the morning of day 3 (the day of ecdysis being termed day 0), when the wheat was replaced with artificial diet. The diet used was the p-diet of Abisgold and Simpson (1987) and contained 14 % protein and 28 % digestible carbohydrate by dry weight. Details of the exact composition and preparation of diets are given elsewhere (Simpson and Abisgold, 1985; Abisgold and Simpson, 1987). The average intermeal interval on this diet is 48 min in insects feeding *ad libitum*.

Locusts were left with the artificial diet for at least 3 h and at most 7 h. At some time during this period they were removed for experimentation after having been observed taking a meal of at least 3 min duration in the course of *ad libitum* feeding (see Abisgold and Simpson, 1987, for further details).

Experiment 1: the role of centrifugal neural feedback

The legs of each locust were restrained by wrapping the thorax in double-sided adhesive tape. The anterio-dorsal part of the head was then waxed to a microscope slide and the rest of the insect was stuck ventral side uppermost to the slide by contact between the wrapping of adhesive tape around the thorax and another piece applied to the slide. This resulted in the head assuming a prognathous position. Dental wax was then applied to immobilize the mandibles and labrum, and both maxillary palps were waxed with their domes pointing anteriorly, so that the gustatory sensilla were available for electrophysiological recording.

Having restrained the insect, the next stage was to expose the suboesophageal ganglion (SOG) and cut the maxillary nerve to one of the palps. The maxillary nerve provides the only route for centrifugal neural modulation of taste receptors on the palp dome. The SOG was exposed by freeing the submentum from the cervical membrane along its length, cutting each of its lateral processes and retracting the labium with a hooked needle mounted in a micromanipulator. The maxillary nerve on one side was then sectioned close to the ganglion. The labium was replaced and Parafilm was stretched across to seal the wound.

Thirty-five minutes after the termination of the last meal on artificial diet, responses to a 0.0125 mol l^{-1} solution of amino acids in 0.05 mol l^{-1} NaCl were recorded from 10 sensilla on the nerve-sectioned palp and a similar number of sensilla on the intact palp. The amino acid solution consisted of a 41:46:33:47:52:46:37:28 ratio of leucine:glutamine:serine:methionine:phenylalanine:lysine:valine:alanine. This is the ratio present in the artificial diet of those amino acids shown to be important in the compensatory response (Simpson *et al.* 1990*b*). Tip-recordings were made in the usual manner (see Abisgold and Simpson, 1988, for details) and traces were stored for later analysis on tape using a

Racal Store 4DS. Each sensillum was stimulated once. The numbers of spikes generated in the first second of stimulation were counted either from the screen of a Tektronix 5223 digitizing storage scope or by using SAPID Tools (Mitchell *et al.* 1990). The first second of stimulation has previously been found to provide similar trends to those obtained by looking at the behaviourally more relevant phasic portion (approximately the first 300 ms) of the response (Simpson *et al.* 1990*a*; J. D. Abisgold and S. J. Simpson, unpublished data).

Forty minutes after the end of the last meal of diet, each insect was injected with 10 μl of either a 0.175 mol l^{-1} mix of amino acids in distilled water or a 0.175 mol l^{-1} solution of xylose in distilled water. The injection was administered between abdominal tergites four and five using a Hamilton syringe. The mix of amino acids was that required to raise the haemolymph profile to that found in insects which had taken a meal on a diet containing 28 % rather than 14 % protein 40 min earlier (Abisgold and Simpson, 1987). The injection contained 134 nmol of threonine, 212 nmol of glutamine, 30 nmol of serine, 267 nmol of methionine, 217 nmol of leucine, 110 nmol of phenylalanine, 133 nmol of isoleucine, 251 nmol of lysine, 111 nmol of valine and 283 nmol of alanine. Xylose was used as the control since it has a similar rate of removal from the blood as does the amino acid mix and it is not utilized by locusts (Abisgold and Simpson, 1987).

Ten minutes after the injection, the responsiveness of a sample of at least 10 hairs was once more tested by stimulation with the 0.0125 mol l^{-1} solution of amino acids in 0.05 mol l^{-1} NaCl. These sensilla were not identified as being the same ones that were stimulated before the injection, although it is almost certain that at least some would have been. Given that there are some 350 hairs on each palp dome, it is extremely difficult to locate the same hairs when the preparation has to be moved on the stage of the microscope to enable each palp to be recorded from in turn. Mean responses for the 10 sensilla for each palp were used in the analyses. The alternative approach is to record from fewer, identified sensilla before and after injection. This has been done in experiment 2 (as it was in Abisgold and Simpson, 1988), where only one palp was recorded from during the experiment.

In our previous work (Abisgold and Simpson, 1988) we found that the injection of amino acids caused a 60–70 % reduction in the response of sensilla to stimulation with the amino acid mix, whereas the xylose injection had no effect. If such a response is mediated *via* centrifugal neural feedback, the reduction following injection of amino acids should only occur in sensilla borne on the palp with its maxillary nerve still connected to the SOG. If both palps exhibit the response, then the effect cannot be neural.

Experiment 2: local versus *central control*

Locusts were restrained for recording as above, except that only one maxillary palp was waxed forward. Prior to waxing, the palp was tightly ligatured with a human hair, so that the terminal two segments were isolated from the rest of the animal. Thirty-five minutes after the end of the last meal, three sensilla were

stimulated with both the 0.0125 mol l^{-1} amino acid mix in 0.05 mol l^{-1} NaCl and a 0.05 mol l^{-1} solution of NaCl alone.

Forty minutes after the meal, the palp was injected with either 0.02 μl of the same amino acid mix as in experiment 1 or with 0.02 μl of the xylose solution. This volume was chosen to cause a similar increase in amino acid profile of the haemolymph of the isolated segments of the palp as would injection of 10 μl into the abdomen were the palp not ligatured, assuming complete mixing of the haemolymph throughout the body. On the basis of the external dimensions of the palp and its internal structure (Blaney and Chapman, 1969), it was estimated that the volume of haemolymph in the terminal two segments of the palp is about 0.4 μl.

The injection was administered *via* a microelectrode (1.5 mm outside diameter, capillary glass) with its tip broken to create a jagged end. The volume of solution was taken up in a 1-μl Hamilton syringe and injected into the shank of the micropipette. Capillarity drew the solution to the tip. A length of cannulation tubing was then forced over the shank end of the micropipette and attached to a 15 ml syringe. The micropipette was placed in a micromanipulator and moved so that the tip penetrated the flexible dome of the palp where it joins the annular ring of hard cuticle on the terminal segment. The tip was advanced to a depth of about one-quarter of the length of the segment, keeping its path parallel with the segment so as not to pierce and enter the large tracheal air sac. The solution was then expelled by depressing the plunger of the syringe.

Five minutes after injection, the same three sensilla were restimulated with 0.0125 mol l^{-1} amino acids in 0.05 mol l^{-1} NaCl and with 0.05 mol l^{-1} NaCl.

If centrally mediated release of hormones is responsible for the modulation of taste responsiveness, the tiny, local injection of amino acids would not be expected to influence gustatory sensitivity.

Experiment 3: the feasibility of local modulation

In experiment 2 it was assumed that amino acids injected into the abdomen reach the tips of the palps and are available to modulate locally the responsiveness of taste receptors. The final experiment was designed to ascertain whether there is a change in composition of the haemolymph in the palp, within the time that the feedback is known to occur following injection of amino acids into the abdominal haemocoel.

Upon completion of a meal, locusts were restrained on a microscope slide with double-sided adhesive tape and wax. Forty minutes after the end of the meal, each locust was injected with three solutions. The total volume injected was 25 μl. The first injection was 5 μl in volume and, except for alanine, contained the amounts of amino acids required to raise the haemolymph profile to that of a locust which had eaten a high-protein meal 40 min earlier. In calculating amounts to inject, account was taken of the fact that blood volume was being raised by 25 μl rather than 10 μl, as in experiment 1. The amount of alanine injected was 5.6 nmol (1.9 %) less than

that needed to raise the haemolymph profile to that of a high-protein-fed insect. This difference was made up in the second injection, which contained 10 μl of L-[U-^{14}C]alanine (specific activity 0.09 mCi ml^{-1} in 0.1 mol l^{-1} HCl solution; Sigma Chemical Co. Ltd), producing 2.0×10^{6} disints min^{-1}. The third injection consisted of 10 μl of a 1 mCi ml^{-1} solution of [methoxy-^{3}H]inulin (specific activity 212 mCi g^{-1}; NEN research products) in autoclaved distilled water, producing 2.2×10^{7} disints min^{-1}.

To prevent leakage, solution 1 was injected between abdominal tergites 4 and 5, while the other two solutions were injected one segment further back.

Either 5 or 10 min after the injections, 0.5–1.5 μl of haemolymph was collected into disposable micropipettes from the maxillary palps, the frons, the coxal membrane of a prothoracic leg and from between abdominal sternites 8 and 9. To collect blood from the palp, the dome was removed and slight pressure was applied to the abdomen. A maximum of 0.6 μl was expelled since removing greater volumes would draw on blood from the cranial haemocoel. Experiments were carried out at either 22 °C (samples collected 5 or 10 min later), the temperature at which electrophysiological recordings were made, or at 30 °C (samples collected 5 min later), the temperature at which the insects were reared and fed.

Haemolymph was expelled onto a square (90 mm×90 mm) of glass microfibre filter paper (Whatman, grade A) and placed in a polyethylene insert within a glass scintillation vial. Samples were dried overnight at 40–50 °C. Approximately 4 ml of scintillant [0.4 g of 2,5-diphenyloxazole, PPO, and 0.01 g of 1,4-bis(5-phenyl-oxazol-2-yl) benzene, POPOP, made up to 100 ml with toluene] was added to each sample. Samples were then counted in an LKB Wallac 125 RackBeta liquid scintillation counter. Values for each isotope were corrected for quenching using previously constructed calibration curves.

Amino acids in the haemolymph are continually exchanged and converted. To establish that the label recovered in the haemolymph after injection was still attached to the alanine molecule, samples of haemolymph were chromatographed on cellulose layers and the position of the label was compared with that of alanine markers. The effect of haemolymph on the movement of alanine was first established with chromatograms of alanine in 75 % ethanol and alanine in ethanol with haemolymph added. Subsequently, [^{14}C]alanine and a sample of blood withdrawn from the palp 10 min after injection into the abdomen of [^{14}C]alanine were chromatographed.

The procedures for running and developing chromatograms were as follows. Samples were applied to one corner of TLC cellulose-coated plastic sheets (20 cm×20 cm, thickness 0.1 mm, Merck) and dried. Plates were placed in a chromatography tank containing a 2:2:1 (v/v/v) solution of chloroform, methanol and 17 % ammonia, until the solvent front had travelled approximately 15 cm. The plates were dried in a forced air draught, turned through 90°, and returned for a second run in 75 % phenol. Plates were removed from the tank after the solvent front had travelled approximately 12 cm. Plates 1 and 2 were treated with ninhydrin spray (0.5 % ninhydrin in butan-1-ol; BDH Chemicals) and warmed on

a hot plate until an even colour developed. X-ray films (Kodak X-Omat XAR 5) were placed over plates 3 and 4, left for 1 week at −70°C, and then developed.

Results

Experiment 1: the role of centrifugal neural feedback

Results are summarized in Table 1 and Fig. 1. Prior to injection of amino acids or xylose, the firing rates of sensilla on the nerve-sectioned and intact palps in response to stimulation with 0.0125 mol l^{-1} amino acids in 0.05 mol l^{-1} NaCl did not differ significantly and provided a combined mean of 35±3.7 spikes s^{-1} (S.E.M.). After an injection designed to raise the profile of amino acids in the haemolymph to that of an insect fed on a high-protein diet there was a marked and statistically similar reduction in responsiveness in sensilla on both palps (15±5.7 spikes s^{-1} reduction for the intact palp and 20±11.1 spikes s^{-1} reduction for the palp with the cut nerve). Such a change was not evident after a control xylose injection (0.1±3.9 spikes s^{-1} reduction and 3±3.6 spikes s^{-1} increase, respectively).

These data both confirm the results of Abisgold and Simpson (1988) and show unequivocally that there is no centrifugal neural feedback modulating taste sensitivity. The effect must, therefore, be haemolymph-borne.

Experiment 2: local versus *central control*

Results are presented in Table 2 and Fig. 2. Before injection, the mean firing rate of sensilla on the ligatured palp was 33±4.4 spikes s^{-1} in response to stimulation with the 0.0125 mol l^{-1} mix of amino acids in 0.05 mol l^{-1} NaCl and 27±4.6 spikes s^{-1} in response to stimulation with 0.05 mol l^{-1} NaCl alone. Follow-

Table 1. *Summary of the ANOVA for experiment 1, in which the nerve to one maxillary palp was sectioned*

Source	Degrees of freedom	Variance ratios for change in firing rate
Injection	1	5.4*
Palp	1	0.4
Injection × Palp	1	0.0
Residual	28	
Total	31	

Injection refers to the treatment involving injection with either amino acids or xylose, while palp is the treatment in which the maxillary nerve was either sectioned or left intact. The palps within an insect are taken as being independent (although the results do not differ if they are considered to be dependent). Results show a significant effect of injecting amino acids *versus* xylose, but no main or interactive effect of sectioning a palp's nerve.

* $P<0.05$.

ing micro-injection of amino acids into the terminal segments of the palp, there was a profound reduction in the responsiveness of the same sensilla to stimulation with amino acids (22±5.5 spikes s^{-1} reduction), but no significant change in the response to stimulation with NaCl alone (6±6.0 spikes s^{-1} decrease). When xylose was injected there was no significant change in the response to amino acids (a 7±3.5 spikes s^{-1} increase) or to NaCl (a 4±9.1 spikes s^{-1} decrease).

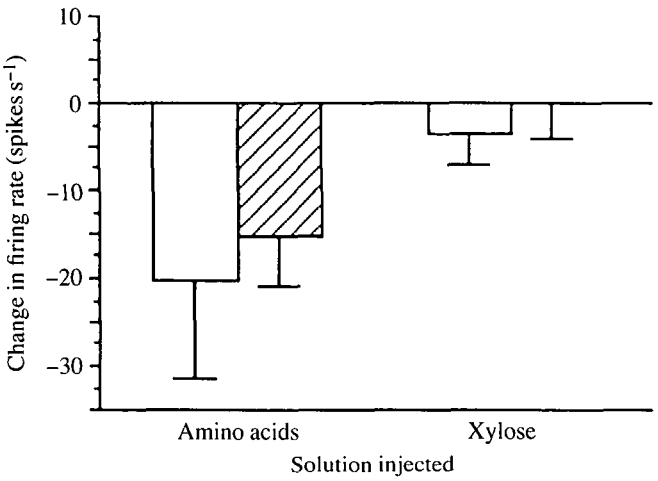

Fig. 1. Histograms showing the mean change in firing rate of maxillary sensilla in response to stimulation with 0.0125 mol l^{-1} amino acids in 0.05 mol l^{-1} NaCl 10 min after an injection of amino acids or xylose into the abdomen. The open bars are for the palp with its maxillary nerve sectioned at the suboesophageal ganglion, while the hatched bars are for the other maxillary palp with its nerve intact. Data for each bar are means + standard errors for the average response for a population of 10 sensilla on each maxillary palp from eight insects. The combined mean firing rate of sensilla on both palps in response to stimulation with amino acids before the injection was 35±3.7 spikes s^{-1}.

Table 2. *Summary of the ANOVA for experiment 2, in which the palp was ligatured and micro-injected with either amino acids or xylose*

Source	Degrees of freedom	Variance ratios for change in firing rate to stimulation with	
		Amino acids	NaCl
Injection	1	17.9***	0.0
Residual	16		
Total	17		

There is a highly significant effect of the nature of the solution injected when sensilla are stimulated with amino acids, but no effect for stimulation with salt.

*** $P<0.001$.

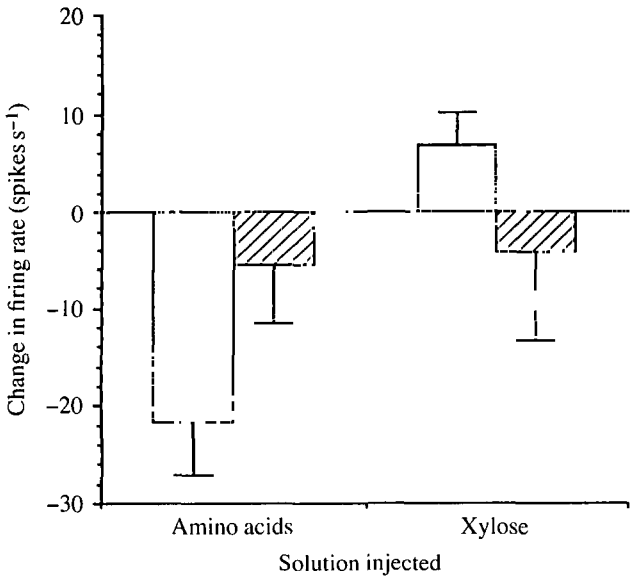

Fig. 2. Histograms showing the mean change in firing rate of maxillary sensilla in response to stimulation with 0.0125 mol l^{-1} amino acids in 0.05 mol l^{-1} NaCl (stippled bars) and 0.05 mol l^{-1} NaCl alone (hatched bars), 5 min after a 0.02 μl injection of either amino acids or xylose into the ligatured terminal two segments of a maxillary palp. Data for each bar are the means + standard errors from nine insects, each insect's contribution to the mean being the average change in firing rate from three identified sensilla stimulated once each with amino acids and salts before, and once after, the injection. The mean firing rates of sensilla before the injection in response to stimulation with amino acids and NaCl alone were 33±4.4 and 27±4.6 spikes s^{-1}, respectively.

These data are very much as expected had the injections been 10 μl in volume and administered in the abdomen (Abisgold and Simpson, 1988), rather than being only 0.02 μl injected into the palp. It is clear, then, that the modulation of peripheral gustatory sensitivity can be mediated locally either at, or close to, the taste receptors.

Experiment 3: the feasibility of local modulation

An injection of amino acids into the abdomen has an effect on the responsiveness of maxillary palp chemosensilla within 10 min. The site chosen for injection was close to the region where nutrients from the gut would be absorbed into the haemolymph across the midgut. Previous work showed that there is a sharp rise in the concentration of free amino acids in the blood of the thorax within the course of a high-protein meal (Abisgold and Simpson, 1987), but is this also apparent for the haemolymph within the terminal part of the palp 10 min later?

The mean number of high-energy emissions (due to the isotope ^{14}C) detected in haemolymph from the palps was higher than background, but not significantly so,

Table 3. *Summary of the ANCOVA for experiment 3 in which [^{3}H]inulin and [^{14}C]alanine were injected into the abdomen and recovered in haemolymph sampled from the palps, head, thorax and abdomen either 5 or 10 min later*

		Variance ratio				
Source	d.f.	Covariate (^{3}H)	Main variate (^{14}C)	d.f.	Regression	Main variate-adjusted means
Time	1	10.3**	8.9*	1	6.8*	0.6
Residual	10			9		
Site	3	12.3***	12.8***	3	53.5***	0.9
Time × Site	3	6.3**	7.3**	3	53.5***	1.4
Residual	30			29		
Total	47					

Analyses are presented for the two isotopes separately using ANOVA and for ^{14}C corrected for ^{3}H (the covariate). The fact that the adjusted means show no significant effect of time or site indicates that the two isotopes are behaving in the same way. Since inulin would not be expected to be taken out of the blood, this suggests that neither is the labelled alanine.

* $P<0.05$; ** $P<0.01$; *** $P<0.001$.

d.f., degrees of freedom.

5 min after injection at an ambient temperature of 22°C (359±80.3 *versus* a background of 216±5.1 disints min^{-1}, $N=5$). However, by 10 min after the injection at 22°C and by 5 min at 30°C the mean amount of high-energy isotope detected was significantly above background ($F_{1,10}=42.1$, $P<0.001$ and $F_{1,10}=6.9$, $P<0.05$, respectively).

Analyses of covariance were used to assess the effects of ambient temperature (22 or 30°C) and the time elapsed since injection (5 or 10 min) on the amount of ^{3}H and ^{14}C recovered from each of the four regions of the body. The results are summarized in Tables 3 and 4 and Figs 3 and 4.

At both temperatures and times the highest concentrations of both isotopes were recovered from the abdomen, smaller but similar levels were found in the thorax and head, and the lowest concentrations were recovered from the palps. The effect of raising the temperature was not particularly marked, although it did, as might be expected, result in a more even distribution of the injected isotopes. Delaying the removal of blood samples by 5 min also led to a more even distribution of ^{3}H from the abdomen to the head and palps. This was not so clearly seen for ^{14}C, as a result of a rather low recovery from the abdomen at 5 min relative to 10 min.

When means for ^{14}C were adjusted for values of ^{3}H (by using ^{3}H as a covariate), the significance of time and site of collection on recovery of ^{14}C disappeared. This result, along with the fact that inulin is not metabolized, indicates that the difference in distribution of the high-energy isotope between the various regions of the body is predominantly due to diffusion and movement of haemolymph rather

Table 4. *Summary of the ANCOVA for experiment 3 in which [^{3}H]inulin and [^{14}C]alanine were injected into the abdomen and recovered in haemolymph sampled from the palps, head, thorax and abdomen 5 min afterwards at an ambient temperature of 22 or 30°C*

		Variance ratio				
Source	d.f.	Covariate (^{3}H)	Main variate (^{14}C)	d.f.	Regression	Main variate-adjusted means
Temperature	1	6.7*	0.4	1	1.8	0.0
Residual	8			7		
Site	3	11.9***	2.7	3	5.6*	0.0
Temperature × Site	3	2.5	0.6	3	5.6*	1.9
Residual	24			23		
Total	39			23		

See caption for Table 3. * $P<0.05$; *** $P<0.001$.
d.f., degrees of freedom.

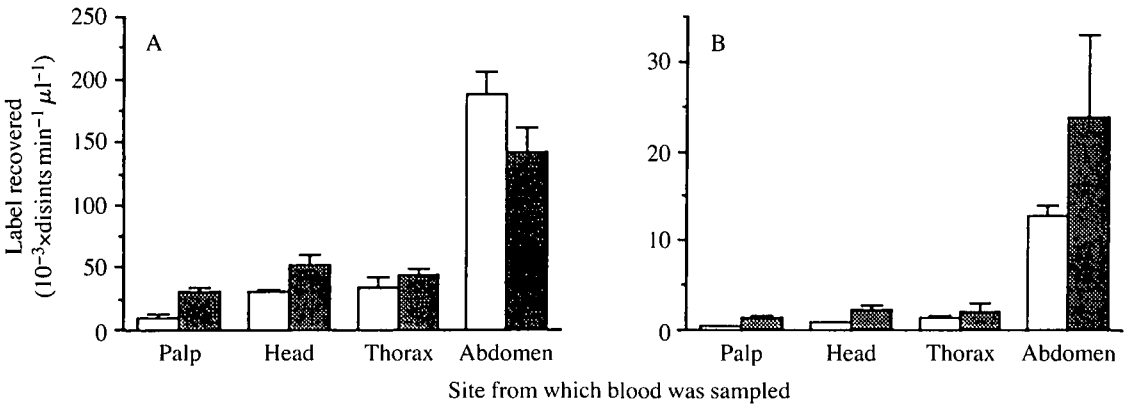

Fig. 3. The concentration of [^{3}H]inulin (A) and [^{14}C]alanine (B) in haemolymph sampled from the four named sites 5 (open bars) and 10 (stippled bars) min after an injection into the abdominal haemocoel. Data are expressed in disintegrations per minute (disints min^{-1}) and are the means + standard error from six insects.

than to the uptake from the blood of labelled alanine between the sites of injection and collection. This was verified by estimating the total amounts of both isotopes present in the haemolymph at the times of sampling. The procedure for doing this was to multiply the concentrations of label recorded for the head, thorax and abdomen by estimates of the blood volume in each of these regions, and then to sum the values. A total of 2.2×10^7 disints min^{-1} of ^{3}H and 2.0×10^6 disints min^{-1} of ^{14}C was injected. Assuming a blood volume of 220 µl (Abisgold and Simpson,

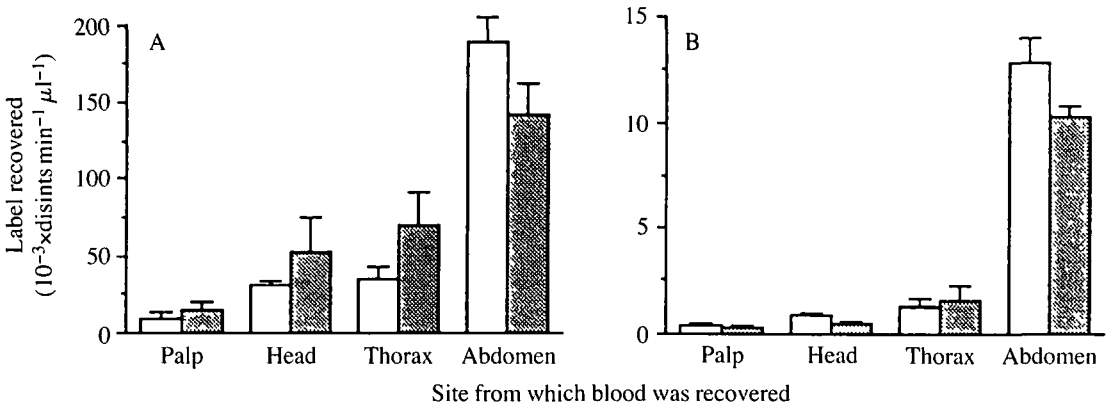

Fig. 4. The concentration of [^{3}H]inulin (A) and [^{14}C]alanine (B) in haemolymph sampled from the four named sites 5 min after an injection into the abdominal haemocoel at either 22°C (open bars) or 30°C (stippled bars). Data are expressed in disintegrations per minute (disints min^{-1}) and are the means + standard error from five insects.

1987), distributed 1:2:2 between the head, thorax and abdomen, then levels of ^{3}H in the haemolymph were 2.1×10^7 and 2.0×10^7 disints min^{-1} at 5 and 10 min after injection, respectively, whereas, at the same times, levels of ^{14}C were 1.4×10^6 and 2.3×10^6 disints min^{-1}.

Absence of any significant removal of the injected amino acid is consistent with the outcome of pilot studies by Abisgold and Simpson (cited in 1987) showing that the expected changes in blood amino acid profiles and osmolality were realized following an amino acid injection. This would not have been the case had the nutrients been removed to the fat body.

Fig. 5A,B shows tracings of the two-dimensional chromatograms of alanine in ethanol, and alanine in ethanol with haemolymph. The addition of haemolymph slowed the migration of alanine in the second solvent. A similar difference existed between the positions of the blackened spots on the autoradiograms of [^{14}C]alanine (Fig. 5C) and blood from animals injected with [^{14}C]alanine (Fig. 5D), indicating that the label was still attached to alanine in the haemolymph circulating in the palp.

While appreciable amounts of alanine are apparent in the haemolymph of the palp within 10 min of an injection into the abdomen, the injected amino acids have not become evenly distributed throughout the body by that time. Using the inulin data (which are probably more reliable than those for alanine, owing to the greater amount of label injected), about 35–40 % of the compound that would have been expected, had the injection become homogeneously distributed in the haemocoel, was recovered after 10 min at 22°C. Using data from Abisgold and Simpson (1987), this would mean that the amino acid concentration present in the blood of

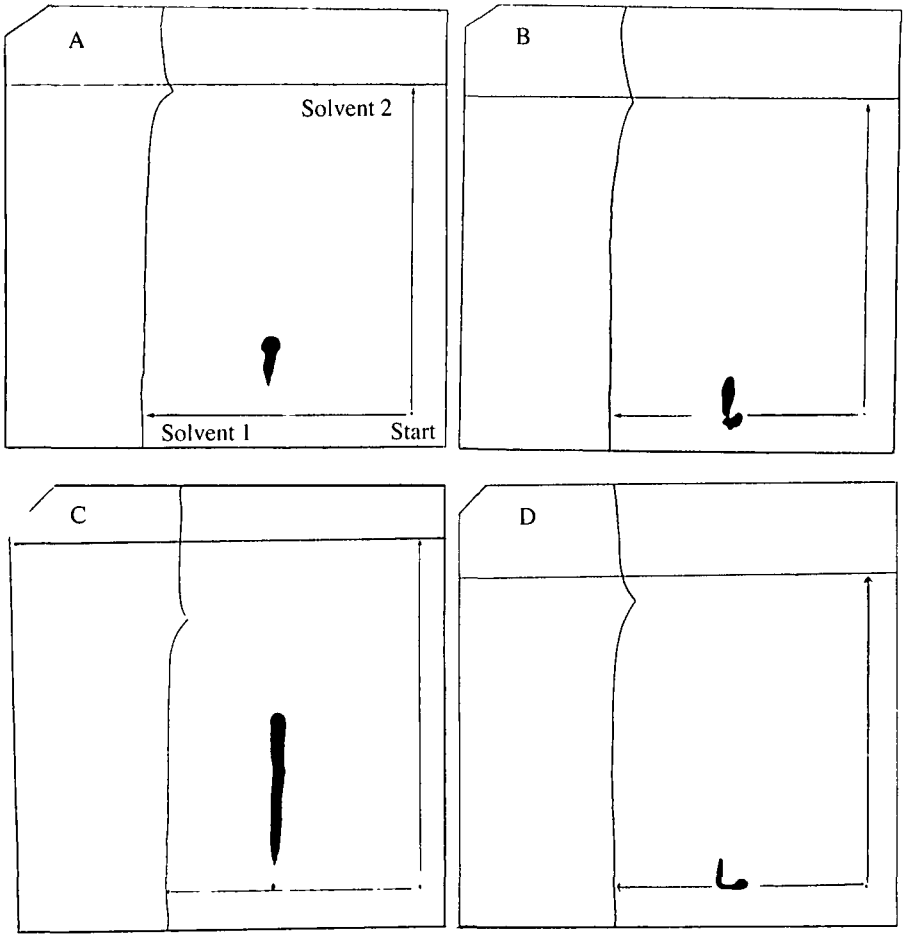

Fig. 5. (A) Trace of a two-dimensional separation of alanine in 75 % ethanol. The plate was dried in a forced draught after development in each direction. The dark spot of alanine was made visible with ninhydrin. Solvent 1 is $CHCl_3$–MeOH–17 % NH_3 (2:2:1 v/v/v) and solvent 2 is phenol–water (75:25 w/w). (B) As for A but with the alanine in 75 % ethanol with haemolymph added. (C) As for A but for [^{14}C]alanine in 0.1 mol l^{-1} HCl. X-ray film was placed over the plate for 1 week at −70°C, then developed. The dark spot is labelled alanine. (D) As for C but for a sample of blood taken from the maxillary palp of a locust 10 min after injection into the abdomen. The black spot is in the same position as in B, indicating that the label is still attached to alanine.

the palp of an insect fed 40 min earlier on the 14 % protein diet would have risen by 25 % in the 10 min after an injection of amino acids into the abdomen.

Discussion

We have previously demonstrated that nutrient-specific feedbacks for sugars and amino acids independently regulate the responsiveness of maxillary palp

chemoreceptors of locusts, and that, for the latter, this feedback is mediated *via* free amino acids in the haemolymph (Abisgold and Simpson, 1988; Simpson *et al.* 1991). The present results indicate that the amino acid feedback acts locally within the palp and does not involve the central nervous system.

Peripheral modulation of sensory responsiveness is increasingly well known in all manner of animal taxa and sensory systems (Pasztor, 1989). Variation in gustatory chemosensitivity with age, developmental stage, time of day and feeding history has been reported in several insect species (see review by Blaney *et al.* 1986; Schoonhoven *et al.* 1991; Simmonds *et al.* 1991). We have found amino acid and sugar feedbacks very similar to those for the locust in caterpillars of *Spodoptera littoralis* (Simmonds *et al.* 1992). Such work was part of a comparative study of the two groups. The mechanisms underlying the effect have not been studied in caterpillars. Nevertheless, the similarity in response between such diverse representatives of the Insecta may indicate that the modulation of gustatory responsiveness by blood metabolites is widespread within the class.

The first experiment in the present paper demonstrated that centrifugal neural control is not involved in the amino acid feedback. Sectioning the maxillary nerve did not prevent the reduction in responsiveness of maxillary palp sensilla to stimulation with amino acids following an injection of amino acids into the abdomen. There has so far been no demonstration of centrifugal control of insect chemoreceptors. Such feedback has been found to occur in vertebrates (Brush and Halpern, 1970; Roper, 1989). Ultrastructural investigations of mouthpart chemosensilla in locusts and cockroaches have shown the presence of what appear to be efferent axonal processes close to the taste receptor cells, indicating that there could be centrifugal control (Cook, 1972; Moulins and Noirot, 1972). If there is, it is not acting in the context of amino acid feedbacks onto maxillary sensilla.

The second experiment showed just as clearly that there is no centrifugal hormonal modulation involved in the amino acid response. Such modulation has previously been found to occur in locusts by Bernays and co-workers (Bernays and Chapman, 1972; Bernays *et al.* 1972), who found that compounds released from the corpora cardiaca as a result of distending the crop during feeding led to increased electrical resistance across the domes of the maxillary palps, probably by causing the terminal pore of each gustatory sensillum to close. However, this is not a nutrient-specific response, but part of the general reduction in behavioural activity that follows any meal. The response could be elicited by cannulating the non-nutritive substance agar into the crop.

Instances of hormonal modulation of insect chemoresponsiveness are known (see review by Blaney *et al.* 1986; den Otter *et al.* 1991). The best understood is the change in response of the lactic acid receptors in olfactory sensilla on the antennae of female mosquitoes, where increases in sensitivity accompanying host-seeking behaviour are associated with juvenile hormone, while decreased responsiveness following blood feeding is caused by a hormone released from the fat body (Davis, 1984; Bowen *et al.* 1988).

There is one report of internal chemoreceptors monitoring the blood of an insect and eliciting release of hormones. Okajima *et al.* (1989) found that saline with a high content of histidine or trehalose elicited increased afferent, and subsequently decreased inhibitory efferent, activity in nerve 4 to the prothoracic gland of larval *Mamestra brassicae*. It was proposed that the afferents monitor titres of nutrients in the blood and ensure the accelerated synthesis or release of ecdysteroid at appropriate stages of growth.

Results of the second experiment indicate that the interaction between amino acids in the haemolymph and taste receptors occurs locally within the maxillary palp itself. The experiment does not allow us to say quite how the interaction is mediated, but there are some likely candidate mechanisms. These fall into two classes. In the first, the amino acids are detected by some local receptor organ, which then modulates the taste receptors. Perhaps the most likely candidate for such an organ would be the accessory cells, which isolate the lumen of the sensillum from the haemolymph and regulate the ionic and chemical composition of the fluid bathing the dendrites of the taste cells (the so-called 'sensillum liquor').

In the second, the nutrients interact directly with the receptors. This would provide the most direct form of control. The simplest possible mechanism would be where the composition of the sensillum liquor reflects that of the haemolymph, and metabolites from the haemolymph bind to the same molecular acceptor sites that receive stimulants from the food *via* the pore at the tip of the sensillum. In this way the receptor cells would act as difference detectors, their threshold to externally applied nutrients being set by chronic adaptation to levels of those same metabolites found in the dendritic liquor.

It is known, at least in blowflies, that the chemical composition of the fluid in the sensillar sinus can correspond to that of the haemolymph through the activities of the accessory cell barrier (Phillips and Vande Berg, 1976). Whether this is also true for locusts and, if so, whether the ciliary sinus, in which the dendrites lie, is also accessible to blood metabolites is not known.

The radio-labelling studies (experiment 3) indicate that appreciable levels of labelled alanine and inulin injected into the abdomen reach the palp within the time taken for an injection of amino acids into the abdomen to induce peripheral changes. Within the injection mix of 10 amino acids only 1.9 % of the alanine was labelled. Nevertheless, the similarity in distribution of labelled alanine and inulin following the injection as well as the fact that the ^{14}C isotope remained attached to circulating alanine confirm that the injected amino acids reach the palps. We have overestimated somewhat the amount injected into the palp in experiment 2, since the labelling studies show that the elevation of amino acid titres in the palp 10 min after an abdominal injection is 35–40 % of that following the micro-injection into the palp. The latter was based on the assumption that complete mixing of the blood within the body would have occurred in the 10 min following an abdominal injection. This discrepancy is not so great as to favour or disfavour any of the mechanisms discussed so far.

A mechanism in which modulation occurs as a result of binding of blood-borne

metabolites to dendritic acceptor sites is particularly appealing. It would provide the necessary specificity of response to account for the independent modulation within a sensillum of responsiveness to amino acids and sugars (Simpson *et al.* 1991). It would even enable different classes of acceptor sites within the same generalist taste neurone to be modulated independently. We have yet to distinguish between the 6–10 individual taste receptors within each maxillary sensillum, so it is not possible to say yet whether modulation for both sugar and amino acid sensitivity occurs within a single cell or whether cells specialize in the compounds for which their responsiveness is modulated (Simpson *et al.* 1991). Work on locusts and grasshoppers suggests that the taste neurones have a rather broad response spectrum (Blaney, 1974, 1975, 1981; White and Chapman, 1990). The situation is further complicated by the fact that interactions occur between salt and both amino acids and sugars in a stimulating solution (Abisgold and Simpson, 1988; Simpson *et al.* 1991).

A direct blood metabolite/dendritic acceptor site mechanism might provide an explanation for the observation that adding an enriching mix of amino acids to a low-protein diet only induces a compensatory reduction in feeding if certain key amino acids are present. Removal of only one of these leads to the diet being eaten in quantities appropriate to the absence of the entire enriching mix (Simpson *et al.* 1990*b*). We found that this behavioural effect was reflected in peripheral modulation. When lysine or alanine (the two most important of the key amino acids in the diet studies) were omitted from an abdominal injection of amino acids, the expected reduction in responsiveness of the maxillary receptors to stimulation with amino acids failed to appear. This could be explained if there are multiple acceptor sites for amino acids on the dendritic membranes, all of which must be filled by blood-borne amino acids to cause reduction in receptor responsiveness. Multiple sites for amino acid transduction apparently occur in other insects (see Van Loon and Van Eeuwijk, 1990).

Such a mechanism can only operate for compounds to which the taste neurones are responsive and which are found both in the haemolymph and in the food. This could apply not just to nutrients such as salts, sugars and amino acids, but perhaps also to certain allelochemicals. This could account for the modulation of sensory responsiveness to such compounds that occurs with feeding history in caterpillars (Schoonhoven *et al.* 1991; Staedler and Hanson, 1976).

There are similarities between the direct blood metabolite/dendritic acceptor site model and two phenomena found in vertebrates. One is 'intravascular taste', where, in humans, injection of sweet- and bitter-tasting substances into the blood elicits the sensation of applying these to the tongue (Fishberg *et al.* 1933). Holland *et al.* (1991) have recently shown in dogs that tastants in the blood can diffuse out and reach the microvilli of the taste receptors on the tongue. The other analogous phenomenon involves the peripheral modulation of salt responsiveness in rats. It has been proposed that part of the modulation may be caused by adaptation of salt receptors on the tongue to the levels of sodium in the saliva that bathes them. Salivary sodium titres reflect the sodium reserves of the body (Contreras and

Frank, 1979; Contreras *et al.* 1984). Other processes such as hormonal control are probably also involved in peripheral modulation of taste responsiveness to salt (Priehs *et al.* 1991).

Whatever the exact mechanism turns out to be, it is clear that peripheral modulation is not the sole controller of nutritional compensation. Learning and direct modulation of behaviour centrally are also involved (Abisgold and Simpson, 1987; Cohen *et al.* 1988; Simpson and Simpson, 1990; Simpson and White, 1990; Champagne and Bernays, 1991). Peripheral modulation can, in theory, provide the basis for the complex behavioural decisions made by locusts in the face of their changing nutritional needs and the variable quality and availability of food (Raubenheimer and Simpson, 1992).

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