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Taste-enhancing Effects of Glycine on the Sweetness of Glucose: a Gustatory Aspect of Symbiosis between the Ant, *Camponotus japonicus*, and the Larvae of the Lycaenid Butterfly, *Niphanda fusca*

Ayako Wada, Yu Isobe[1], Susumu Yamaguchi[2], Ryohei Yamaoka and Mamiko Ozaki

Department of Applied Biology, Faculty of Textile Science, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, [1]Department of Biology, Faculty of Science, Nara Women's University, Nara 630-8506 and [2]205 Lakeside, 3-2-8 Ishikami-chyo, Nerima-ku, Tokyo 177-0041, Japan

Correspondence to be sent to: Mamiko Ozaki, Department of Applied Biology, Faculty of Textile Science, Kyoto Institute of Technology, Kyoto 606-8585, Japan. e-mail: mamiko@ipc.kit.ac.jp

Abstract

The lycaenid butterfly, *Niphanda fusca*, has a parasitic relationship with its host ant, *Camponotus japonicus*: the caterpillars may use chemical mimicry to enter the ant nest where they are fed mouth-to-mouth by the adult ants until pupation. Nevertheless, larvae offer their host ants a nutritious secretion that contains 160 mM glucose and 43 mM glycine. Using glucose and glycine mixture as artificial secretions, we investigated the gustatory effect of glucose and/or glycine on the ants. Glycine induced neither feeding behavior nor gustatory response in the ants if its concentration was $<$500 mM. In the presence of glycine at the concentration in the secretion, however, the ants improved their preference to glucose, and the sugar receptor cell exhibited electrophysiological enhancement of response to glucose in a glycine-concentration-dependent manner. By adding glycine to glucose in their secretions, therefore, the butterfly larvae can manipulate the gustatory sense of the ants. The alluring taste of 'glycine-flavored glucose' could motivate the host ants to feed the larvae and thereby receive the secretions as a reward. The taste enhancement created by the combination of sugar and amino acid may play a role in the evolution of the parasitic relationships of these insects. The taste-enhancing effect appears to be analogous to taste enhancement by 'umami' substances in humans.

Introduction

The caterpillars of many species of lycaenid butterflies associate with ants and these interactions can range from parasitism to mutualism (Atsatt, 1981; Henning, 1983; Pierce, 1987; Thomas *et al.*, 1989; Hölldobler and Wilson, 1990; Fiedler *et al.*, 1996). One of the representative species in Japan, *Niphanda fusca* (Nagayama, 1950), remarkably and in contrast to most other ant-parasitic lycaenids, feeds on aphid honeydew instead of plants when young. They start to produce a sweet secretion from a specialized endocrine gland called the dorsal nectary organ (DNO) at the third instar stage. The ants, *Camponotus japonicus*, carry the larvae of this age to their nests and care for them over the winter months. The ants have a strong interest in the larval DNO secretions while feeding and grooming (see Figure 1A). They often tap on the back of the larva to stimulate secretion from the DNO (see Figure 1B). Once an ant has taken a drop of secretion, it holds it with its labial and maxillary palps (arrows in Figure 2A) and shares it with other nestmates. Thus, the DNO secretion is thought to be crucial in retaining the relationship between the butterfly larvae and the ants (Wardlaw *et al.*, 2000). However, these behavioral observations have not been coupled with studies of the sensory physiology of the ants and how they perceive the taste of the secretion.

The secretions of *N. fusca* have only once analysed because of difficulty in capturing the larvae and in collecting the small amounts of secretion. The analysis of Nomura *et al.* (Nomura *et al.*, 1992) showed that the secretion contains 160 mM glucose (75% of the total sugar) and 43 mM glycine (76 % of the total amino acid). It has been reported that the larvae of other myrmecophilous butterflies, *Jalmenus evagoras* (Pierce, 1989; Pierce and Nash, 1999) and *Lysandra hispana* (Maschwitz *et al.*, 1975) secrete serine and methionine instead of glycine, respectively (see Discussion). As for the sugar component, a separate study found that the secretion of *N. fusca* contained 600 mM glucose (45% of the total sugar) (D. Chogyoji, personal communication). This difference in concentration of glucose may be due to differences in the nutritional states of the colonies of host ants feeding the larvae of *N. fusca*.

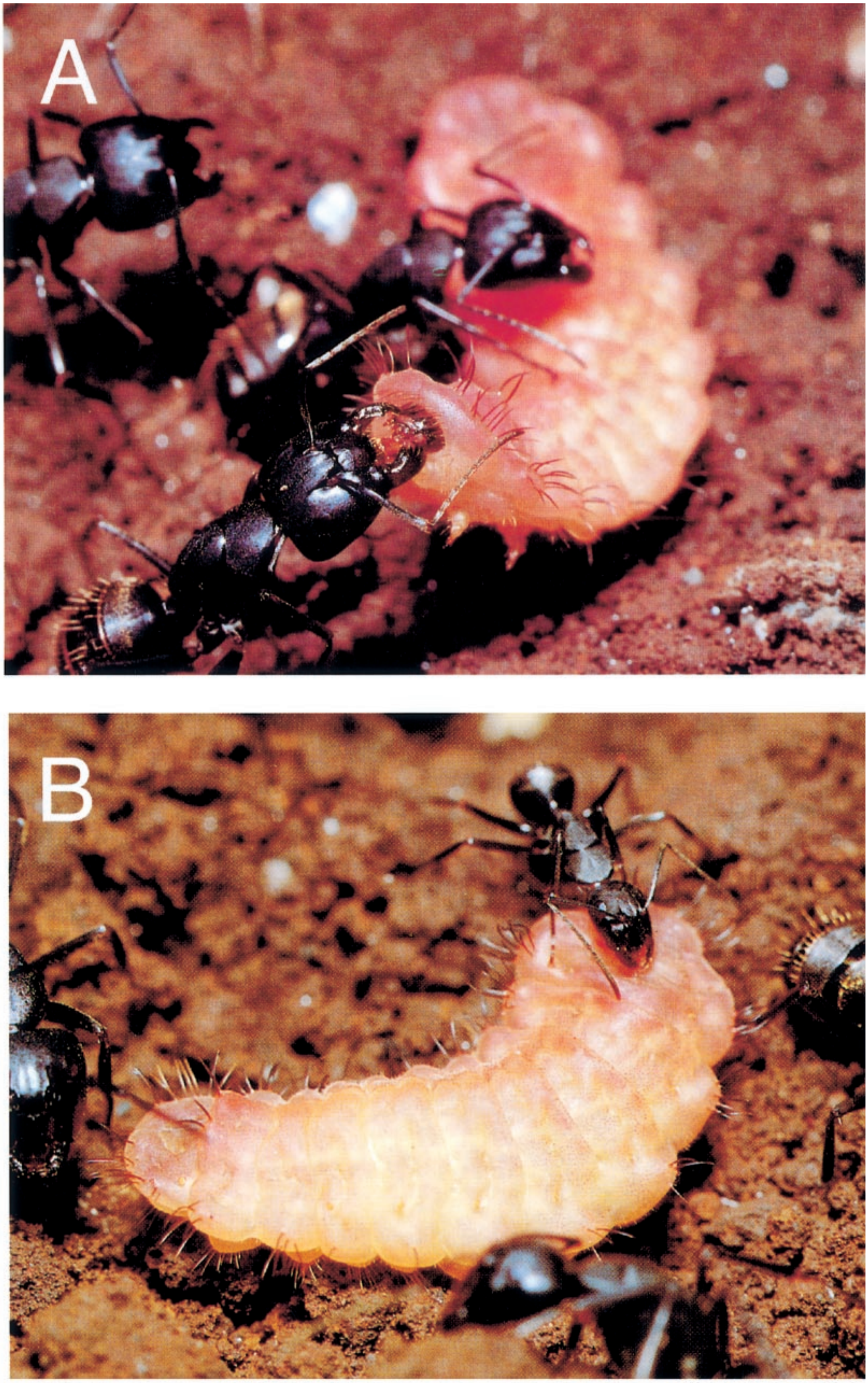

Figure 1 The ant, *C. japonicus*, and the larvae of the lycaenid butterfly, *N. fusca*. **(A)** An ant is feeding a larva of the lycaenid butterfly. **(B)** An ant tapping on the back of a larva to stimulate the DNO into producing a secretion.

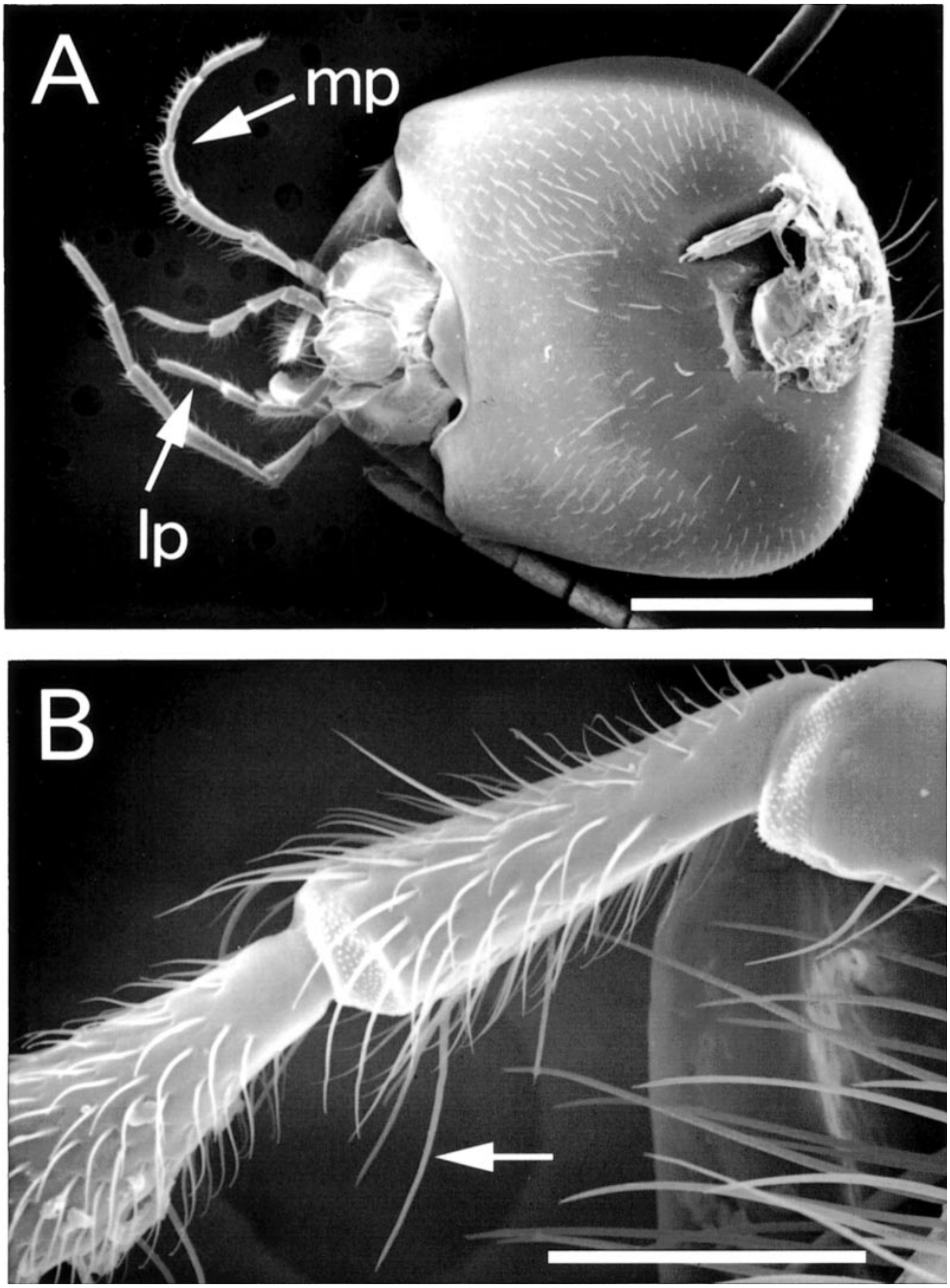

Figure 2 Scanning electron microscope image of the taste sensilla of the ant. **(A)** Dorsal view of the head of a worker: mp, maxillary palps; lp, labial palps. The scale bar indicates 1 mm. **(B)** High magnification of a labial palp. The arrow indicates the taste sensillum used for electrophysiological experiments. Scale bar = 0.1 mm.

Based on these data, we conducted behavioral and electrophysiological experiments on the taste of the DNO secretion of *N. fusca*, hypothesizing that the glucose–glycine combination in the DNO secretion could provide an especially alluring taste for workers of *C. japonicus*.

Materials and methods

The ants

The ants, *C. japonicus* (Hymenoptera: Formicidae), were all collected from the same colony in the field. In our laboratory, they were kept in plastic boxes (350 × 225 × 55 mm) with only a supply of water. We subjected them to electrophysiological experiments 2 days after collection. Six days after collection, we used them for the behavior experiments, because the period of food deprivation increases the feeding sensitivity to be analysed. As the median of feeding threshold, $K_{1/2}$, differed among groups of ants (see Figures 4A and 5A), data from a series of behavioral experiments were obtained in the same population.

Behavioral tests

We conducted two types of behavior tests: (i) choice-and-drink tests to examine preference between two kinds of test solutions and (ii) feeding response tests to determine the feeding threshold of a test solution.

The choice-and-drink test was carried out according to the method of Tanimura *et al.* (Tanimura *et al.*, 1982), which was originally designed for fruit flies. In order to determine the relative effects of glucose and glucose plus glycine, we used three test plates, each of which had 60 wells. In each plate, the 60 wells were alternately filled with 10 μl of glucose solution and glucose-plus-glycine solution. In plate 1, the glucose solution was stained with a blue food color (brilliant blue FCF) and the glucose-plus-glycine solution was colorless, so that the blue and the colorless wells made a checkered pattern. In plate 2, colorless glucose and blue glucose-plus-glycine solutions were used. In plate 3, both solutions were colorless. In preliminary experiments, we established that the blue food color used in our study affected neither electrophysiological nor feeding response to glucose. We placed four ants onto each of these three plates and let them drink freely for 3 h in the dark. The ants were then frozen and their crops were isolated. The crops were homogenized and extracted with 1 ml of 50 % ethanol. The absorbance of the extracts was measured at 630 nm with a spectrophotometer. In order to eliminate non-specific absorbance by the intrinsic crop extract, the absorbance of the stomach extract in the ants of plate 3 was subtracted from the absorbance of the crop extract of the ants of plates 1 and 2. Using the compensated absorbancies of the extracts of plates 1 (Abs.1) and 2 (Abs.2), the preference rates for glucose and glucose-plus-glycine solutions were defined as Abs.1/(Abs.1 + Abs.2) and Abs.2/(Abs.1 + Abs.2), respectively. If Abs.1/(Abs.1 + Abs.2) = Abs.2/(Abs.1 + Abs.2), the ants showed no difference between glucose and glucose plus glycine. If Abs.1/(Abs.1 + Abs.2) > Abs.2/(Abs.1 + Abs.2), the ants preferred glucose to glucose plus glycine. If Abs.1/(Abs.1 + Abs.2) < Abs.2/(Abs.1 + Abs.2), the ants preferred glucose plus glycine to glucose. Furthermore, we carried out similar experiments using methionine and serine instead of glycine, because the major amino acid in the DNO secretion is glycine in *N. fusca*, but serine has been reported to be the main amino-acid component of the DNO secretion in *Jalmenus evagoras* (Hunt *et al*., 1982) and methionine has been found in trace amounts in the DNO secretion of *Lysandra hispana* (Maschwitz *et al.*, 1975).

For the feeding response tests, a glucose concentration series of the test solution was prepared (10 concentration steps using 1:2 dilutions with distilled water, starting from 2 M glucose) and three other glucose concentration series were prepared in the same way, but using 1:2 dilutions with 50, 100 or 200 mM glycine. Twenty ants were randomly chosen from a single colony and each was placed in a plastic pipette tip with the top cut to fit the head of the ant, so that the head of the ant stuck out of the opening. Thus restrained, the ants in the pipette tips were convenient to handle under the binocular microscope. A drop of test solution was placed under the microscope and carefully touched with the maxillary and the labial palps. Observing movements of the glossa, we quickly judged whether the ant was prepared to drink the test solution or not. We then counted the number of ants showing this feeding response with glossa extension. In drawing a concentration–feeding response curve based on this kind of experiment, the number of ants showing glossa extension was plotted against the logarithmically scaled concentration of glucose and curve fitting was done by computer.

Electrophysiological procedure

The isolated head of the ant was connected to an indifferent platinum electrode. A glass capillary containing the stimulus solution also contained the platinum wire of the recording electrode. Under the microscope, the long chemosensillum on the second segment of the labial palps (arrow in Figure 2B) was focused and the capillary was slipped over the tip of the sensillum. Thus, the sensory response was simultaneously recorded when the stimulus touched the sensillum tip (Hodgson *et al.*, 1955). The stimulus solutions were prepared with various concentrations of glucose and/or glycine dissolved in 100 mM NaCl. The 100 mM NaCl solution alone did not induce significant responses (see Figure 6B). The duration of stimulation was 0.7–1 s and intervals of at least 3 min between stimuli were taken to avoid any effects of adaptation. We used only workers with responsive sensilla for these assays; a sensillum was considered to be responsive if it generated >9 impulses/0.5 s to glucose in 100 mM NaCl.

Scanning electron microscope

The isolated heads of the ants were mildly sonicated in distilled water, dehydrated through the ethanol series of 70, 80, 85, 90, 95, 99 and 100% and finally treated with 100% acetone. They were then transferred into iso-amyl acetate. After they had been dried in the critical-point dryer, each specimen was put on a sample stage, coated with gold and observed with a scanning electron microscope (Hitachi S-2100A).

Results

Effects of glycine on glucose-feeding behavior

In the choice-and-drink experiment (Figure 3), we tested 400 mM glucose versus 400 mM glucose plus 50 (A), 100 (B), or 200 mM glycine (C). This concentration of glucose was the average value of measurements by Nomura *et al.* (Nomura *et al.*, 1992) and D. Chogyoji (personal communication). The results showed that the preference for glucose alone decreased from 0.35 ± 0.04 (n = 4) to 0.09 ± 0.07 (n = 4), whereas the preference for glucose plus glycine increased from 0.65 ± 0.04 (n = 4) to 0.91 ± 0.07 (n = 4), as the concentration of glycine was increased from 50 to 200 mM. When we used 200 mM fructose instead of 400 mM glucose, taste enhancement was also observed in the presence of 100 mM glycine. We also examined the effects of 100 mM serine (E) and 100 mM methionine (F) on feeding preference for glucose in *C. japonicus*. However, serine and methionine did not affect the preference for glucose in *C. japonicus*. As shown in Figure 4, 400 mM glucose induced feeding in several sets of 20 ants, but <500 mM glycine did not induce feeding in any ants from the same colony.

In Figure 5, the percentage of ants showing a feeding response is plotted against the concentration of glucose in the presence of 0 (A), 50 (B), 100 (C) or 200 mM glycine (D). The concentrations of glucose at which 50 % of the ants showed the feeding response, $K_{1/2}$, were 490, 320, 220 and 100 mM in the presence of 0, 50, 100 and 200 mM glycine, respectively. Statistically, the distribution of the feeding threshold is regarded as a logarithmic normal distribution, so that the value of $K_{1/2}$ corresponds to the median of the feeding threshold in a test population (Hirakawa and Kijima, 1978). Thus, glycine, even at concentrations that are ineffective in eliciting feeding in the absence of glucose, significantly decreases the feeding threshold of the ants to glucose, resulting in an increase of their preference rate for glucose plus glycine, as shown in Figure 3.

Effects of glycine on the sugar taste response of the chemosensillum

The electron micrograph of Figure 2A shows the dorsal view of the head of *C. japonicus*. Pairs of maxillary and the labial palps are seen (arrows in A). At high magnification (B), an arrow indicates a long type of sensillum located on the second segment of the labial palps. From this type of sensillum, we recorded sensory responses. The ants use the palps to share droplets of the DNO secretion.

In preliminary observations by scanning electron microscopy, we found several types of chemosensilla in the mouthparts. Among them, an elongate taste sensillum on the second segment of the labial palps was the most reliable in retaining normal function under laboratory conditions. Therefore, we investigated the effects of glycine on the electrophysiological response of this sensillum.

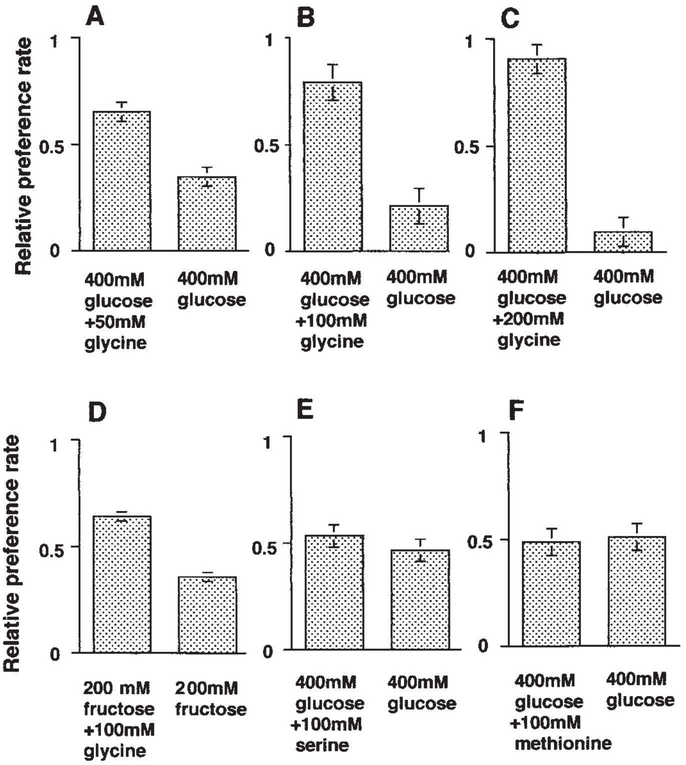

Figure 3 Feeding preference for glucose in the absence or presence of glycine. The preference for 400 mM glucose plus **(A)** 50, **(B)** 100, or **(C)** 200 mM glycine is compared with that of 400 mM glucose alone in a sample of ants. The preference for 200 mM fructose plus 100 mM glycine **(D)** is compared with that for 200 mM fructose alone. The preference for 400 mM glucose plus (D) 100 mM glycine, **(E)** serine, or **(F)** methionine is compared with that for 400 mM glucose. The error bars indicate the standard deviations (n = 4).

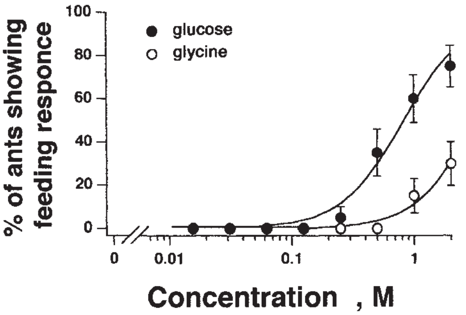

Figure 4 Concentration–feeding response relationships for glucose and glycine. The percentages of ants showing feeding responses are plotted against the logarithmically scaled concentrations of glucose and glycine, respectively. The curve fittings correspond to with the equations $y = 100x^{1.6}/(K_{1/2}{}^{1.6} + x^{1.6})$ and $y = 100x^{1.2}/(K_{1/2}{}^{1.2} + x^{1.2})$ on the concentration–response relationships for glucose and glycine, respectively. The error bars indicate standard deviations (n = 20).

First, we recorded sensory responses from this sensillum to 500, 100 and 10 mM NaCl (Figure 6). Stimulation with 500 mM NaCl induced impulses of large amplitude (A), but stimulation with 100 (B) or 10 mM NaCl (C) did not. These impulses were derived from a salt receptor cell. Stimulation with 100 or 10 mM NaCl induced impulses of small amplitude, which were derived from a water receptor cell (dots). As is characteristic of impulses from water receptor cells, the frequency decreased as the concentration of NaCl increased.

Since 100 mM NaCl only induced a small number of impulses from the water receptor cell, we used 100 mM NaCl to dissolve sugars and/or glycine for stimulus solutions. When the sensillum was stimulated with 400 mM glucose dissolved in 100 mM NaCl (D), a few impulses from the water receptor cell (dots) were also seen, but all other impulses differed in amplitude from impulses typically observed in either water or salt receptor cells. We concluded that these impulses of middle amplitude were derived from the sugar receptor cell. The absolute amplitudes of impulses of the salt (to 500 mM NaCl), the water (to 100 mM NaCl) and the sugar receptor cells (to 400 mM glucose in 100 mM NaCl) are listed in Table 1.

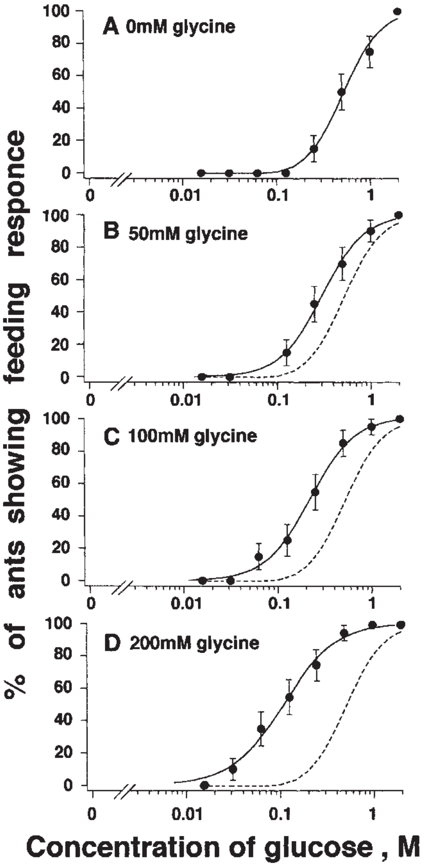

Figure 5 Effect of glycine on feeding responses to glucose. The percentage of the ants showing feeding response is plotted against concentration of glucose in the presence of **(A)** 0, **(B)** 50, **(C)** 100 and **(D)** 200 mM glycine. The curve fittings correspond to the equations, $y = 100x^{1.8}/(K_{1/2}^{1.8} + x^{1.8})$ for (A–C) and $y = 100x^{1.6}/(K_{1/2}^{1.6} + x^{1.6})$ for (D). The curve in (A) is indicated by broken lines in (B–D). The error bars indicate standard deviations (n = 20).

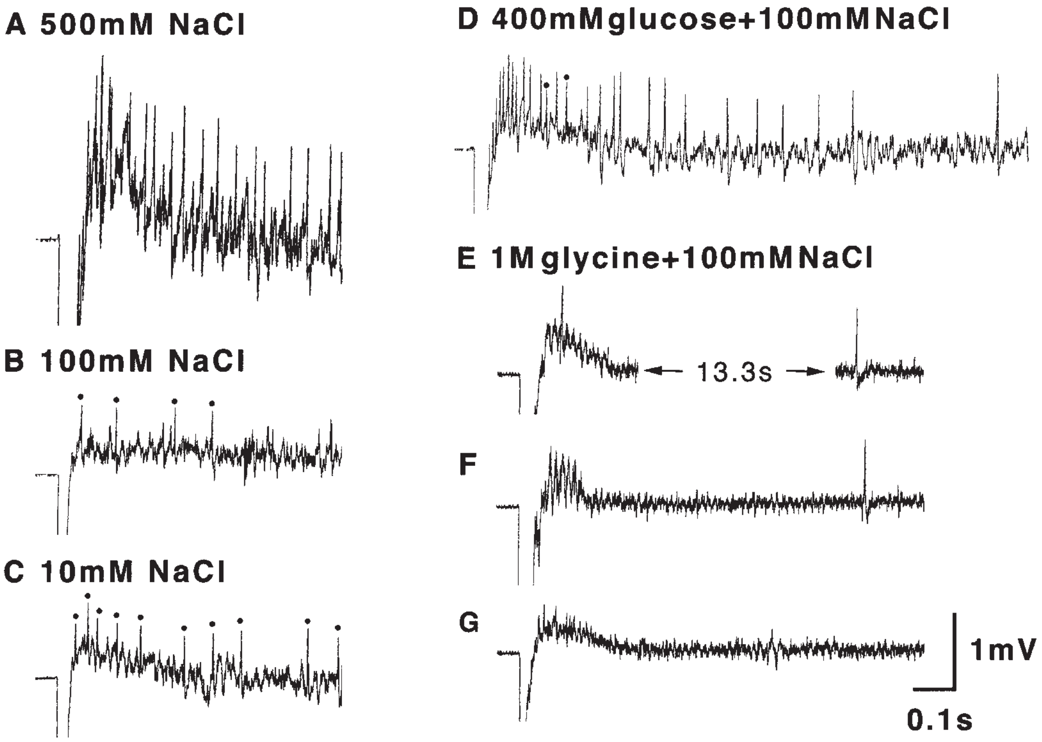

Figure 6 Impulse recordings of the responses in the sensillum on the labial palps. The impulses elicited by: **(A)** 500, **(B)** 100 and **(C)** 10 mM NaCl; **(D)** 400 mM glucose; and **(E–G)** 1 M glycine dissolved in 100 mM NaCl are seen. The impulses of the water receptor cell are indicated by dots.

Table 1 Amplitudes of impulses

Test solutions (receptor cell)	Average impulse amplitude ± SD (mV)	No. of impulses measured
500 mM NaCl (salt)	1.45 ± 0.12	18
100 mM NaCl (water)	0.74 ± 0.06	12
400 mM glucose + 100 mM NaCl (sugar)	1.17 ± 0.10	13

One mole of glycine dissolved in 100 mM NaCl induced, in <20 % of the trials, a small number of impulses from the sugar receptor cell (E, F). In most cases, however, glycine induced no impulses (G) or occasionally a small number of impulses of the water receptor cell (data not shown). In Figure 6, the impulses in A–C were recorded in the sensillum of one ant and those in D-G in different sensilla of another ant.

As shown in Figure 6, 1 M glycine typically did not induce electrophysiological responses from the sugar receptor cell (G), but, rarely, such responses were observed (E, F). Therefore, the sugar receptor cells in the sensilla on the labial palps may have responsiveness to glycine, but this response may be masked because the threshold for glycine is higher than that for glucose. Even if the ants have other types of taste organs sensitive to glycine than the sensilla examined here, their threshold for glycine would still be higher than that for glucose, because Figure 4B shows that only 15% of ants tested responded to 1 M glycine.

In order to investigate the concentration-dependent effect of glycine on the taste reception of glucose, sensory responses were recorded to 100 mM NaCl solutions of 400 mM glucose plus 0 (A), 50 (B), 100 (C) and 200 mM glycine (D), respectively (Figure 7). We observed that the impulses of the sugar receptor cell increased as the concentration of glycine increased, although the tested concentrations of glycine on its own did not excite the sugar receptor cell. We counted the number of impulses generated for 0.5 s (between black arrows in Figure 7) starting from 0.1 s after the beginning of stimulation. The same experiment was repeated in the same type of sensillum in four different animals, as shown in the diagram of Figure 8. The magnitudes of response to 400 mM glucose plus 100 or 200 mM glycine are significantly different from those to 400 mM glucose alone (Student's *t*-test, $P < 0.05$).

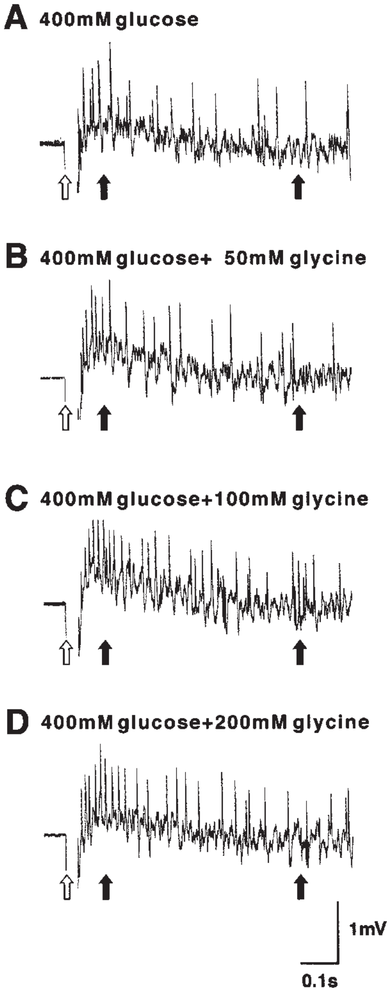

Figure 7 Impulse recordings of the response to glucose in the presence of glycine. The impulses elicited by 400 mM glucose in the presence of **(A)** 0, **(B)** 50, **(C)** 100 and **(D)** 200 mM glycine are seen. The white arrow indicates the beginning of stimulation. The black arrows at 0.1 and 0.6 s mark the interval for which the number of impulses was counted for Figures 8 and 9.

Figure 9A shows the glucose concentration–response curves in the absence and presence of 200 mM glycine. The relative magnitudes of response normalized to the response to 1 M glucose in the absence of glycine are plotted. We could not confirm whether the maximum response to glucose was changed by glycine or not, because the responses to concentrations of glucose >1 M were reduced in their amplitudes for unknown reasons and were, therefore, difficult to count. After stimulation with high concentrations of glucose, the responsiveness of the sensilla tended to be unstable, so that 32 sensilla examinations resulted in only three complete pairs of concentration–response curves comparable in the same sensilla. However, in the presence of 200 mM glycine (closed circles), the concentration–response curve appeared to shift to the left of the control curve in the absence of glycine (open circles). Since 200 mM glycine itself did not induce any impulses of the sugar receptor cell, it would appear that glycine has a synergistic rather than an additive effect on the response to glucose.

Discussion

Role of amino acid in DNO secretion

Myrmecophilous lycaenid butterflies have a wide range of life cycles. Some species, such as *Jalmenus evagoras* from Australia, feed on foliage throughout their larval stages. The larvae and pupae offer attendant ants nutritious secretions

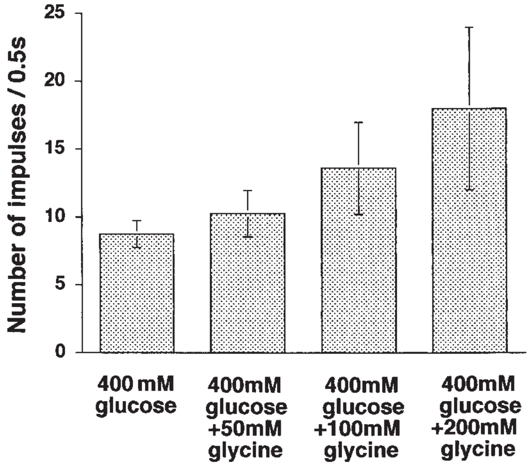

Figure 8 Effects of glycine on the taste response to glucose. The impulses of the sugar receptor cell generated for 0.5 s starting from 0.1 to 0.6 s after beginning of stimulation with 400 mM glucose plus 0, 50, 100 or 200 mM glycine are compared with each other. The error bars indicate standard deviations (n = 4).

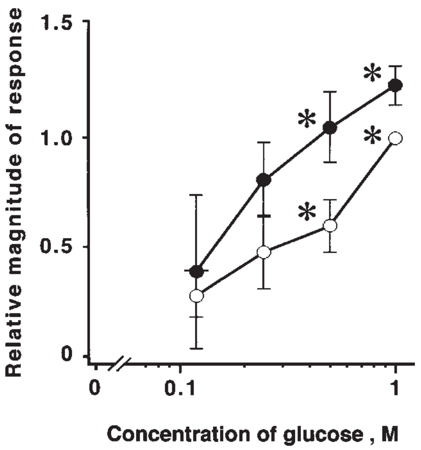

Figure 9 Effect of glycine on the concentration–response relationships for glucose. The numbers of impulses of the sugar receptor cell generated for 0.5 s starting from 0.1 to 0.6 s after beginning of stimulation were normalized to that of 1 M glucose in the absence of glycine and plotted against concentration of glucose in the absence (open circles) and presence (closed circles) of 200 mM glycine. The error bars indicate standard deviations (n = 3). Asterisks indicate significant differences between the presence and absence of 200 mM glycine (Student's t-test, $P < 0.05$).

and, in return, the ants protect them against parasites and predators (Pierce, 1984, 1987; Pierce and Nash, 1999). Other species, such as *Maculinea arion*, begin feeding on flowers, but after the third instar, drop to the ground where they are picked up by ants and are carried into the nest. They feed on the ant brood until pupation (Hölldobler and Wilson, 1994). *Niphanda fusca*, a Japanese myrmecophilous lycaenid butterfly, is likewise a parasite of the attendant ants, inducing their host ants, *C. japonicus*, to feed them mouth-to-mouth by trophallaxis inside the nest. The larvae of *N. fusca* are completely dependent upon their host ants for food. Nevertheless, why should they simultaneously reward ants with secretions from the DNO?

Although the DNO secretion may include other substances than sugars and amino acids, studies on larval attractiveness to ants have focused on amino acids, because the larvae of lycaenid species that feed on nitrogen-fixing plants are much more likely to associate with ants than other lycaenid species that feed on non-nitrogen-fixing plants (Pierce, 1985; Fiedler, 1995, 1996). The DNO secretions of some myrmecophilous lycaenid butterflies include particularly concentrated amino acids; their content is often an order of magnitude more than that found in most extrafloral nectaries or in honeydew of aphids (Pierce and Young, 1986; Pierce, 1987; Fiedler and Maschwitz, 1989; DeVries, 1991; Fiedler and Saam, 1995). The concentrated amino acids in the DNO secretions have been considered to be an important nitrogen source for the attendant ants. *Camponotus japonicus* cannot detect concentrations of glycine <500 mM (Figures 4 and 6), but ants attracted by the DNO secretion can take up glycine, which could be a metabolically inexpensive nitrogen source for them. Some social insects are known to use their larvae as a kind of collective stomach. The adult workers feed complex proteins to the brood. The brood digest these proteins and then regurgitate solutions rich in free amino acids back to the workers (Hunt *et al.*, 1982). The workers can then use these amino acids as precursors for the many chemicals used in communication and/or metabolic processes. It is possible that the larvae of *N. fusca* use the secretions from the DNO as a means of mimicking the ant brood.

Camponotus japonicus did not respond to glycine, serine or methionine at the concentrations in the DNO secretion reported for *N. fusca* (Nomura *et al.*, 1992) (Figure 4), *Jalmenus evagoras* (Hunt *et al.*, 1982; Pierce, 1989) or *Lysandra hispana* (Maschwitz *et al.*, 1975). However, *C. japonicus* obviously prefer 'glycine-flavored glucose', which mimics the sugar–amino acid mixture in the DNO secretion of *N. fusca*, to plain glucose (Figure 3A–C). *Lysandra hispana* is a facultative and unspecific myrmecophile, whose ant visitors cover a range of ant taxa (Fiedler, 1990). This contrasts sharply with the situation found in *Jalmenus evagoras*, where the caterpillars do have a specific relationship with only a small number of *Iridomyrmex* species and records of associations with other ant species are from highly exceptional situations (Eastwood and Fraser, 1999). Therefore, while in the specific myrmecophile *Jalmenus evagoras* one might expect adaptation of secretions to the taste of the specific host ant, such a specialization would be extremely unlikely in the opportunistic myrmecophile *Lysandra hispana*. On the other hand, different species of ants are known to be able to detect and prefer some amino acids to others (Lanza, 1991; Lanza *et al.*, 1993). The species of ants tending both *Jalmenus evagoras* and *Lysandra hispana* do not, however, include *C. japonicus* and thus would be expected to differ in their gustatory responses. Although we found that the Japanese species of ants *Formica japonica* and *C. obscripes*, which are not the attendant ants of *N. fusca*, would not prefer the 'glycine-flavored glucose' to glucose alone, further comparisons between *C. japonicus*, the attendant ant of *N. fusca*, and the attendant ants of *Jalmenus evagoras* or *Lysandra hispana* should clarify the question of whether different kinds of amino acid increase feeding preference in different species of ant.

Sweet-taste enhancement by amino acid in the ant

In mammals, synergistic taste-enhancing effects of 'umami' substances are well known. This is dramatically observed in synergistic interactions between amino acids and nucleotides, whose tastes are classified into the fifth fundamental taste, 'umami' (Yamaguchi, 1967, 1991). 'Umami' substances result in increase in the palatability of foods and an improvement of appetite in humans; similarly the taste-enhancing effects of glycine on the sweetness of glucose result in an improvement of appetite in the ant, *C. japonicus* (Figure 3).

Taste organs of insects have a unit structure containing a mechanoreceptor and four taste receptor cells that differ in their functions. The salt and the water receptor cells would not trigger feeding responses, as long as insects appropriately maintain intrinsic salt and water balance. The so-called 'fifth cell' or 'fourth taste receptor cell', for which adequate stimuli are not conclusively determined, appears to respond to alkali halides such as CsCl (Gillary, 1966), bitter substances such as quinine (Liscia and Solari, 2000) and lipophilic substances such as limonene (M. Ozaki, unpublished data). These stimuli do not induce feeding responses, but rather inhibit them and sometimes evoke aversion behavior in the blowfly (Dethier, 1976; Liscia and Solari, 2000; Nakamura, *et al*., 2000). Thus, even if the ants have this type of 'fifth cell', it is unlikely to be involved in glucose taste enhancement by glycine. The taste receptor cell, excitation of which induces feeding response to sugars and other nutritious substances such as amino acids, is traditionally called the sugar receptor cell in insects. Because neither glucose (Figure 6D) nor glycine at high concentration (E, F) induced feeding response and evoked impulses of the sugar receptor cell, it is suggested that the taste-enhancement mechanism by glycine in *C. japonicus* is exclusively controlled by the sugar receptor cell.

In insects, the responsiveness of the sugar receptor cells to amino acids depends upon the species and their feeding style. The tsetse fly, for example, can respond to various amino acids (Van der Goes van Naters and Den Otter, 1998), but the fleshfly responds to some (Shimada, 1975; Shimada and Isono, 1978). The blowfly responds to various sugars, but few amino acids (Goldrich, 1973). *Camponotus japonicus* may be more like the blowfly than the tsetse fly or the fleshfly in the low responsiveness of its sugar receptor cell to glycine (Figure 6E–G) and other amino acids (data not shown). For enhancing the taste of glucose, however, glycine need not act as a gustatory stimulus by itself, because even at lower concentrations than the electrophysiological threshold, glycine enhanced the taste of glucose (Figures 7 and 8). In other words, the glycine receptor molecule would not mediate this taste enhancement.

The concentration–response curve shift observed in the presence of glycine (Figure 9) might be explained by an increase in stimulus affinity, the available number of receptor molecules or the efficacy of some steps of the intracellular transduction processes. As we have shown in the present study, however, 200 mM glycine enhanced taste responses in *C. japonicus*, not only to glucose but also to fructose (Figure 3). Considering the multiple-site hypothesis, exemplified by the coexistence of different receptor molecules for glucose and fructose on the sugar receptor cell in the fleshfly (Shimada *et al*., 1974) or the blowfly (Hara, 1983), taste enhancement by glycine in *C. japonicus* is presumed not to be a receptor-molecule-specific event. In order to clarify the precise mechanism of this taste enhancement, however, the target of glycine should be determined at the molecular level after characterization of receptor molecules and transduction mechanisms in the sugar receptor cell of the ants.

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