The Effects of Amino Acids on the Labellar Hair Chemosensory Cells of the Fly

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ABSTRACT The effects of amino acids on the labellar hair chemosensory cells were examined with two kinds of flies (the fleshfly, Boettcherisca peregrina, and the blowfly, Phormia regina). As a result of this examination, the effects of amino acids were divided into four main classes. Amino acids in class 1 did not stimulate any chemoreceptor cell. Amino acids in class 2 inhibited nonspecifically the discharges from three kinds of chemosensory cells. Amino acids in class 3 stimulated the salt receptor cell. Amino acids in class 4 stimulated the sugar receptor cell. A possibility that a fourth neuron in the labellar hair chemosensory cell might be a protein or an amino acid receptor cell was eliminated.

INTRODUCTION

Several papers have been published dealing with the effects of proteins and amino acids on the labellar and tarsal hair chemosensory cells of the fly. In 1961 Dethier (1961) showed by his behavioral experiment that the blowfly reacted specifically to stimulation by certain proteins and that the reaction resulted from a taste mechanism and not from olfaction. He also succeeded in recording from the labellar hair chemosensory cells the electrical responses to protein stimulation.

Wolbarsht and Hanson (1965) reexamined the results of Dethier and made another attempt to examine the stimulating effects of various kinds of amino acids by performing both behavioral and electrophysiological experiments. These authors thought that low molecular weight proteins or amino acids might be concerned directly with the stimulation of protein sensitivity, and therefore, that the use of amino acids as stimuli might provide a clue to a simplification of the results. However, they were unable to obtain positive results from stimulation with amino acids. In the present work, experiments using 19 kinds of amino acids were carried out, and the effects of these amino acids on the labellar hair chemosensory cells were divided into 4 main classes.
MATERIALS AND METHODS

Males of two species of flies, the fleshfly, *Boettcherisca peregrina*, and the blowfly, *Phormia regina*, raised in our laboratory, and ranging in age from 6 to 10 days, were used throughout the present experiments. In the case of the fleshfly, the spikes from the sugar receptor cell were the largest among those from three receptor cells (sugar, salt, and water receptor cells). On the other hand, spikes from salt receptor cells in the blowfly were the largest among those from the three receptor cells. The discharge frequency of the water receptor cell of the blowfly is higher than that of the fleshfly (cf. Figs. 1 and 2). It has been shown (Shiraishi and Morita, 1969) that there are fundamentally no differences between the receptor cells of the blowfly and those of the fleshfly. Therefore, the fleshfly was used for the experiments relating to the sugar receptor cell, and the blowfly was used for the experiments relating to the salt and water receptor cells.

Recently, evidence has been accumulating which shows that two cells, rather than one, characteristically respond to monovalent salts (cf. Dethier and Hanson, 1968). But the recognized facts concerning the second salt receptor cell or the fifth cell named by Dethier and Hanson are not enough to understand its biological meaning, and the fundamental character of the cell which can be clarified by electrophysiological methods. Therefore, only the responses from the classical salt receptor cell, which was thoroughly studied by Evans and Mellon (1962) and by Gillary (1966a, b, c), were used in testing the effects of amino acids.

The methods of preparation, recording, and stimulation were, in principle, the same as those described elsewhere (Morita, 1959; Morita and Shiraishi, 1968; Shiraishi and Morita, 1969). The largest hairs of the labellum which conformed to the classification of Wilczek (1967) were used. Impulses from the chemosensory cell were recorded from a cracked part of the sidewall of the hair by means of a glass capillary electrode, while stimulus solutions were applied to the tip of the hair with another glass capillary. Waterhouse's saline (Buck, 1953) was used as an electrolyte solution in the recording electrode.

Molarity was used for the concentration of the stimulus solution. The duration of the stimuli was less than 0.4 sec, and the response magnitude was defined as the number of impulses during a period of 0.2 sec starting at 0.15 sec after the beginning of the stimulus (stationary period of responses). The intervals between stimuli were suitably chosen for various stimulus materials. For example, the intervals between stimuli for sugar receptor stimulation were 3 min in the cases of sucrose and amino acid concentrations less than 0.2 molar, 4 min for concentrations between 0.2 and 0.4 molar, and 5 min above 0.4 molar.

Ambient temperatures in the course of experiments were 25°C ± 0.2°C. Relative humidities of the experimental room were between 62 and 72% throughout this work and did not change more than 3% in one series of experiments. All amino acids were kindly provided by Azynomoto Co., Inc., Japan, and all were the L type. The pH values of test solutions were from 3 to 7. For example, those of class 2 amino acids are shown in Table I. Within the range of those pH values, the responses of three kinds of
Table I

THE pH VALUES OF CLASS 2 AMINO ACIDS.
HITACHI-HORIBA pH METER, TYPE F-5, WAS USED

| Amino acid    | pH  | Concentration | Temperature °C |
|---------------|-----|---------------|----------------|
| Aspartic acid | 3.09| A saturated solution | 25             |
| Glutamic acid | 3.36| A saturated solution | 25             |
| Histidine-HCl | 4.01| 0.5 molar solution  | 25             |
| Arginine-HCl  | 5.38| 0.5 molar solution  | 25             |
| Lysine-HCl    | 5.68| 0.5 molar solution  | 25             |
| (Distilled water) | (5.80) |                | (25)          |

Chemosensory cells are not influenced by pH (cf. Gillary, 1966 a; Shiraishi and Morita, 1969).

Results

Responses to Water, Salt, Sugar, and Amino Acids

Fig. 1 shows responses from one chemosensory hair of the fleshfly to four different stimuli. As described under Materials and Methods, spikes from the sugar receptor cell of the fleshfly are the largest among all receptor cells. Record C in Fig. 1 shows this. The spikes (record B in Fig. 1) caused by stimulation with 0.125 molar phenylalanine are the same height as those using 0.02 molar sucrose (record C in Fig. 1); therefore, it was concluded that the sugar receptor cell was stimulated by phenylalanine.

Fig. 2 shows similar records from one chemosensory hair of the blowfly on stimulation by four different stimuli. As shown by record B in Fig. 2, the salt receptor cell produced the largest spikes among all the receptor cells. The responses to stimulation by 1 molar proline show the same spike height as those to 0.3 molar NaCl; therefore, it was concluded that the salt receptor cell was stimulated by proline. The water receptor cell of the blowfly (record A in Fig. 2) discharged many more spikes than that of the fleshfly (record A in Fig. 1).

Classifications of the Effects of Amino Acids on the Labellar Hair Chemosensory Cells

Experiments were performed with 19 different L type amino acids divided into 4 different classes according to their effects on the chemosensory cells. Table II shows these classifications of amino acids with the side chain corresponding to each amino acid (cf. Greenstein and Winitz, 1961).
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Class 1. Amino Acids

The amino acids in class 1 did not stimulate any receptor cell of the labellar chemosensory hair. The results from glycine stimulation will be described as representative of the amino acids in class 1. Fig. 3 shows the result from the water receptor cell after stimulation by various concentrations of glycine. The concentration of glycine was increased serially by a factor of two up to a concentration of 1 molar. The circles in Fig. 3 show the magnitudes of the responses which were normalized so that the value of the response to distilled water was unity. The number attached to each circle shows the order of application, and the missing numbers (1, 2, 3, 6, 9, etc.) indicate the application of distilled water. There were no differences between the magnitude of the response to any concentration of glycine and that to distilled water. Therefore, it was concluded that glycine did not affect the water receptor cell. If glycine had been able to stimulate the other receptor cells (salt or sugar receptor cells), responses from these receptor cells should have been recorded at the same time in this experiment but these responses could not be observed.

Since glycine might have an inhibitory influence on the salt or sugar receptor cells, two kinds of experiments were designed. Glycine was dissolved into 0.3 molar NaCl to make a mixture of 1 molar glycine and 0.3 molar NaCl. Less concentrated test solutions were prepared by diluting with 0.3 molar

![Figure 1. Response to (A) H₂O; (B) 0.125 molar phenylalanine; (C) 0.02 molar sucrose; (D) 1 molar NaCl. Botcherisca peregrina.](image)
NaCl, while as a control, plain 0.3 molar NaCl was used. One of the results is shown in Fig. 4. The normalized responses appear to be irregular fluctuations about one.

With similar methods the effects of glycine on the sugar receptor cell were examined. A mixture of 1 molar glycine and 0.1 molar sucrose was prepared and then diluted with 0.1 molar sucrose to produce a less concentrated test solution. As the control, 0.1 molar sucrose was used. One of the results is also shown in Fig. 4. There were almost no differences between the responses to the mixture and those to 0.1 molar sucrose. It was concluded from these results that glycine does not inhibit the sugar and salt receptor cells. Similar results were obtained for the other amino acids of class 1.

**Class 2. Amino Acids**

Class 2 amino acids at high concentrations inhibited all three receptor cells and elicited an off response from the salt receptor cell. As an illustration the inhibitory effects of histidine·HCl will be described. Fig. 5 summarizes the normalized responses from water, salt, and sugar receptor cells to histidine·HCl. As the concentration of histidine·HCl in the stimulus solution was increased, discharges of the water receptor cell were progressively inhibited until complete inhibition was reached at 0.3 molar histidine·HCl.

In the case of the salt receptor cell, test solutions were derived by diluting a
0.5 molar histidine·HCl and 0.3 molar NaCl mixture with 0.3 molar NaCl. Histidine·HCl alone at high concentrations appears to stimulate slightly the salt receptor cell. The results are shown by the dotted line in Fig. 5. Part of the inhibition by histidine·HCl may result from competition between histidine·HCl and NaCl molecules for the receptor site.

### Table II

| Class 1 Amino Acids | Class 2 Amino Acids |
|--------------------|--------------------|
| Glycine            | Aspartic acid      |
| Alanine            | Glutamic acid      |
| Serine             | Histidine          |
| Threonine          | Arginine           |
| Cystine            | Lysine             |
| Tyrosine           |                   |

As to the sugar receptor cell, histidine·HCl was dissolved into 0.1 molar sucrose to make a mixture of 0.5 molar histidine·HCl and 0.1 molar sucrose. Other test concentrations were made by diluting the original solution with 0.1 molar sucrose. The solid circles on the X axis in Fig. 5 show that plain 0.5 molar histidine·HCl does not stimulate the sugar receptor cell.

Therefore it was concluded that histidine·HCl nonspecifically inhibited all receptor cells. However, the details of the inhibitory influence of histidine·HCl seem to differ from cell to cell. The other amino acids in class 2 showed a similar tendency.
Figure 3. Response of the water receptor cell on stimulation by various concentrations of glycine. The response is expressed relative to that to distilled water. The number attached to each circle shows the order of application. Missing numbers (1, 2, 3, 6, 9, etc.) show applications of distilled water. Usually, the stimuli were applied in an order of ascending stimulus concentrations. The following method for the calculation of relative response magnitude applies for all the other figures unless otherwise stated. The relative magnitude of response was calculated by dividing the magnitude of the response to amino acid stimulation by the average response to control stimulation (in this case, distilled water). *Phormia regina.*

Figure 4. The effect of glycine on the salt receptor cell; response from the salt receptor cell to various concentrations of glycine in 0.3 molar NaCl. Solid circles show the response to the mixture of glycine and 0.3 molar NaCl. The response was plotted relative to that to 0.3 molar NaCl. The effect of glycine on the sugar receptor cell; response from the sugar receptor cell to the mixture of 0.1 molar sucrose and various concentrations of glycine. Solid circles show the response to the mixture, which was expressed as relative to that to 0.1 molar sucrose. Both results were obtained from different preparations.
Class 3. Amino Acids

Only 2 amino acids, proline and hydroxy-L-proline, among 19 different species of amino acids belonged in this category. Fig. 6 shows the responses from the salt receptor cell to two different stimuli (NaCl and proline) and the inhibition of the water receptor cell discharge. When the salt receptor cell was stimulated by a low concentration of proline, both the salt and water receptor cells dis-

![Figure 5. Inhibition by histidine·HCl of water, salt, and sugar receptor cells, respectively. Open circles show the response from the water receptor cell to stimulation with plain histidine·HCl. Crosses show the response from the salt receptor cell on stimulation with mixtures of 0.3 molar NaCl and various concentrations of histidine·HCl. The dotted line shows the response from the salt receptor cell to plain histidine·HCl. Solid circles show the response from the sugar receptor cell to mixtures of 0.1 molar sucrose and various concentrations of histidine·HCl. Solid circles on the X axis indicate that the sugar receptor cell was not stimulated by 0.5 molar histidine·HCl. Three sets of data from water, sugar, and salt receptor cells were obtained from different preparations and each set of responses was expressed relative to that of each control stimulus solution (distilled water, 0.1 molar sucrose, and 0.3 molar NaCl, respectively). The solid lines were fitted by eye.

charged impulses. Each symbol bearing the same number identified impulses obtained with the same stimulus since salt receptor spikes could be discriminated from water receptor spikes. A 0.0625 molar NaCl solution was used as a control since it produced nearly the same number of spikes as the maximum response to proline stimulation.

The response-concentration curve for the salt receptor cell produced by NaCl stimulation is rather difficult to understand. The discharge from the salt receptor cell is inhibited by NaCl concentrations in the neighborhood of 0.0156 molar. High concentrations of NaCl produced correspondingly higher
responses. Detailed analysis of this phenomenon was performed by Hori. In contrast, the response-concentration curve for the salt receptor cell produced by proline stimulation is simple. The response magnitude simply rose with increase in the concentration of proline. The water receptor cell was inhibited more strongly by NaCl than by proline. The stimulating effect of hydroxy-L-proline on the salt receptor cell was weaker than that of proline.

Class 4. Amino Acids

The amino acids belonging to class 4 stimulated the sugar receptor cell of the fly. Detailed experiments were performed using phenylalanine and valine. The effects of both amino acids on the sugar receptor cell were very similar. Therefore, only the results for phenylalanine stimulation will be described.

The response-concentration curves for four different stimuli (phenylalanine, sucrose, glucose, and fructose) were compared. The data for the sugar receptor response-concentration curves for phenylalanine, sucrose, fructose, and glucose stimulation were obtained in three series of experiments in which phenylalanine was compared with only one of the sugars at one time. The data presented in Fig. 7 represent an average of three sets of data for each sugar. Therefore, the response-concentration curve for phenylalanine stimulation is an average of nine sets of data; whereas the response-concentration curves for sugar stimulation are derived from three sets of data. All responses were normalized so that the maximum response to phenylalanine stimulation was unity.

1 Hori, N. 1970. Data to be published.
The average threshold values for each stimulus were 0.0001 molar for phenylalanine, 0.001 molar for sucrose, 0.015 molar for fructose, and 0.03 molar for glucose. The relative values of the average maximum response were 1 for 0.01 molar phenylalanine stimulation, 1.2 for 1 molar fructose stimulation, 2 for 2 molar glucose stimulation, and 3 for 2 molar sucrose stimulation. The threshold value for phenylalanine stimulation is very low compared with those for sugar stimulation.

As previously described (Morita and Shiraishi, 1968; Omand and Dethier, 1969) the sugar receptor site of the labellar chemosensory cell of the fly has been thought to consist of two subunits (fructose and glucose subunits). Two molecules of monosaccharides or one molecule of disaccharide are thought to conjugate with each receptor site. From the response curve in Fig. 7 and from further analysis of these curves, the action of phenylalanine on the receptor site was thought to be almost the same as that of the monosaccharides. Investigations of the relationship between the two subunits of the receptor site and the stimulus effects of the phenylalanine molecule seemed to be very interesting.

Glucose was dissolved into 0.01 molar phenylalanine to make a mixture of 0.01 molar phenylalanine and 0.15 molar glucose. The response from the sugar receptor cell to 0.15 molar plain monosaccharides (glucose and fructose) was the same as the maximum response to phenylalanine (cf. Fig. 7). Test solutions were derived from the original solutions by diluting with 0.01 molar...
phenylalanine. Fig. 8 shows the response-concentration curve for the glucose-phenylalanine mixture (open circles) and plain phenylalanine (solid circles). Fig. 9 shows a similar result for the experiment with the fructose-phenylalanine mixture.

**Figure 8.** Responses from a single sugar receptor cell to a mixture of 0.15 molar glucose and various concentrations of phenylalanine and to plain phenylalanine. Open and solid circles show the relative response to the mixture and to the plain phenylalanine, respectively. Responses were normalized with respect to the response to 0.15 molar glucose. Each solid line was fitted by eye.

**Figure 9.** Responses from a single sugar receptor cell to a mixture of 0.15 molar fructose and various concentrations of phenylalanine and to plain phenylalanine. Open and solid circles show the relative response to the mixture and to the plain phenylalanine, respectively. Responses were normalized with respect to the response to 0.15 molar fructose. Each solid line in the figure was fitted by eye.

If the receptor site for phenylalanine were completely independent of those for the monosaccharides, the response elicited by the mixtures would show a tendency to be a summation of the responses to the plain phenylalanine and monosaccharides (especially, in the case of the dilute stimulus solutions).
However, as is apparent from Figs. 8 and 9, this is not the case. In the case of the mixture of fructose and phenylalanine, the responses were inhibited to a greater extent than were those for the mixture of glucose and phenylalanine.

Finally, the effects of pH on phenylalanine were examined in the alkaline pH region (cf. Shiraishi and Morita, 1969). Fig. 10 shows the pH-inhibition curves for 0.01 molar phenylalanine and for 0.1 molar sucrose. As the control stimulus 0.1 molar sucrose at neutral pH was used. The influence of pH on the effectiveness of 0.1 molar phenylalanine resembled that of fructose. Therefore, it was concluded that the phenylalanine molecule had a greater affinity for the fructose subunit than for the glucose subunit.

![Graph showing pH-inhibition curve](image)

**Figure 10.** The pH-inhibition curve of the response of the sugar receptor cell to 0.01 molar phenylalanine and to 0.1 molar sucrose, control. The number attached to each symbol represents the order of stimulation. The solid line was fitted by eye.

**DISCUSSION**

There seem to be two possible reasons for the negative results reported by Wolbarsht and Hanson (1965). The first reason may be concerned with the age of the experimental animal. Their flies were younger than those used in the present experiments. Responses to amino acids could be easily recorded from the flies from 6–10 days old. The second reason may involve the fact that stimulus solutions were a mixture of L and D type amino acids. Only L type amino acids were used in our experiments. As pointed out by Kaneko (1938, 1939) in human beings amino acids of the D type caused a sweet taste, while L type amino acids or naturally occurring derivatives caused a bitter disagreeable taste. It was concluded that taste was related directly to the stereostructure of the compound. Amino acids of both the L and D types in the same test solutions might lead to ambiguous results.

Dethier (1961) indicated from his behavioral and electrophysiological ex-
periments that the blowfly (*Phormia regina*) could discriminate among water, sucrose, and protein, respectively, and that these discriminations, in part, could be performed at the receptor level of the chemosensory cell of the legs and the mouth part. In other experiments, we recorded responses from the sugar and water receptor cells on stimulation with a homogenized bovine liver solution. As shown in Table II a specially differentiated chemoreceptor for amino acids or proteins could not be found in the present experiments although only long chemosensory hairs at the margin of the labellum were studied.

Halpern et al. (1962) studied the taste responses of the rat to glycine and DL-alanine stimulation with electrophysiological and behavioral methods and concluded that these amino acids must be related to the sugar receptor of the rat.

Tateda and Hidaka (1966) made detailed experiments on rats using α-amino acids and sugars, and confirmed this possibility. However, glycine and L-alanine did not stimulate any receptor cell of the fly as shown in Table II. They also explained the response-concentration curve for glycine by assuming a multimolecular combination of the stimuli with the receptor site (four to five molecules per receptor site). In the case of the sugar receptor cell of the fly, it was postulated that at least two molecules of amino acids are necessary to stimulate one receptor site.

Wolbarsht and Hanson (1965) considered the possibility of synergistic action between amino acids and other organic compounds at the receptor cell of the fly. In this connection, detailed analyses were performed using amino acids and sugars. However, this phenomenon was not observed. In the case of the rat taste receptor, a synergistic increase in response was observed for a mixture of sucrose and glycine (Tateda and Hidaka, 1966).

It would be very interesting to clarify the relationship between the effects of amino acids on the labellar chemosensory cell of the fly and the chemical nature of the amino acids. However, in the present experiments, it was very difficult to clarify these relationships (see Table II).

Among amino acids in class 1, glycine and alanine have a small uncharged side chain which does not contain any polar groups, but serine, threonine, and tyrosine have a large uncharged polar side chain.

Amino acids in class 2 have an acidic or basic character. Since a few amino acids were neutralized by HCl, but since one or two of the ionizing groups of one molecule should ionize, the factors which produced the inhibitory effects on the labellar chemosensory cells of the fly could not merely be attributed to their acid or basic character, but seemed to depend on the complicated chemical structure of each amino acid.

Amino acids in class 3 have a heterocyclic ring. Usually the salt receptor cell of the fly is stimulated by a monovalent cation, in which, of course, the
ammonium ion [(NH₄)⁺] is included. Since amino acids have an amino group in their structure, it would seem reasonable to expect that each amino acid would stimulate the salt receptor cell; however, only two amino acids did.

Amino acids in class 4 have a large, uncharged, and nonpolar side chain. Among them, valine, leucine, and isoleucine have an aliphatic side chain. As previously described, serine and threonine which had no effect on all three receptor cells have aliphatic side chains but their side chains contain a polar group (hydroxyl group). In this connection, the presence or absence of the polar group in a molecule seems to exert great influence on stimulus effects of the amino acids on the chemoreceptor cell of the fly.

**Table III**

| Amino acid          | Taste for man | Response in fly | Amino acid          | Taste for man | Response in fly |
|---------------------|---------------|-----------------|---------------------|---------------|-----------------|
| Glycine             | Sweet         | Class 1         | L-Valine            | Bitter        | Class 4         |
| L-Alanine           | Sweet         | Class 1         | L-Leucine           | Bitter        | Class 4         |
| L-Serine            | Sweet         | Class 1         | L-Isoleucine        | Bitter        | Class 4         |
| L-Threonine         | Sweet         | Class 1         | L-Methionine        | Bitter        | Class 4         |
| L-Proline           | Sweet         | Class 3         | L-Phenylalanine     | Bitter        | Class 4         |
| L-Hydroxyproline    | Sweet         | Class 3         | L-Tryptophan        | Bitter        | Class 4         |
| L-Lysine·HCl        | Sweet         | Class 2         | L-Arginine·HCl      | Bitter        | Class 2         |
| L-Citrulline        | Sweet         | Class 2         | L-Arginine         | Bitter        |                 |
| L-Glutamine         | Sweet         | Class 2         | L-Ornithine         | Bitter        |                 |
| L-Aspartic acid     | Sour          | Class 2         | L-Histidine         | Bitter        |                 |
| L-Glutamic acid     | Sour          | Class 2         | L-Histidine         | Bitter        |                 |
| L-Histidine·HCl     | Sour          | Class 2         | L-Histidine         | Bitter        |                 |
| L-Asparagine        | Sour          | Class 2         | L-Histidine         | Bitter        |                 |

Yoshida et al. (1966) divided 25 L type amino acids into 4 main classes on the basis of their taste for man (sweet, sour, bitter, and palatable, respectively). The classification of the tastes of the amino acids for man was found to be correlative with the classification of the effects of these acids on the chemoreceptor cells of the fly. These relationships are summarized in Table III. It is a remarkable point that all amino acids which have stimulating effects on the labellar sugar receptor cell of the fly taste bitter to man.

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