The Stimulating Effect of Fatty Acids and Amino Acid Derivatives on the Labellar Sugar Receptor of the Fleshfly

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ABSTRACT Seven D-amino acids, including D-valine, D-phenylalanine, D-leucine, D-isoleucine, D-tryptophan, D-methionine, and D-a-aminobutyric acid, are markedly less stimulative than the corresponding L-isomers that can stimulate the labellar sugar receptor of the fleshfly. A distinct effect of length of the amino acid side chain is clearly observed. Esterification and amidation of the α-carboxyl group, as well as substitution by hydroxyl and methyl groups, result in extremely decreased responses. Amino acids whose amino groups are located at a position other than the alpha are almost ineffective. With all these rigid stereospecificities of the sugar receptor for amino acids, certain replacement of the α-amino group with the hydroxyl or carbonyl group shows a slight increase of the response at neutral pH. Furthermore, certain fatty acids can stimulate the sugar receptor once the solutions are buffered at neutral pH. This observation was further supported by the presence of a remarkable similarity of stimulating effectiveness between amino acids that can stimulate the sugar receptor and those fatty acids. The similarity was shown by testing the response concentration relationships, the stimulating effect of fatty acid derivatives, the effect of treatment with p-chloromercuribenzoate, the behavioral response, and so on.

INTRODUCTION
Considerable evidence has recently been accumulated, supporting the suggestion that individual chemoreceptors of insects may be sensitive to a broader spectrum of chemicals than was formerly believed possible. For example, the labellar sugar receptor of the blowfly as well as that of the fleshfly is sensitive to a variety of sugars (Dethier, 1955; Evans, 1963; Pflumm, 1972), cyclitols (Jakinovich and Agranoff, 1972), amino acids (Shiraishi and Kuwabara, 1970; Goldrich, 1973), and glycosides (Hanamori et al., 1972). This is not to say that the classic water, sugar, and salt receptors are nonspecific. The requirements for stimulation, on the contrary, are still very restrictive, but are met by more than one category of compounds (e.g., sucrose and leucine for the sugar receptor; Dethier, 1974).

On the other hand, different sites in the sugar receptor of the fly have been suggested, on the basis of both behavioral and electrophysiological studies (Dethier, 1955; Evans, 1963; Morita and Shiraishi, 1968; Omand and Dethier, 1969; Jakinovich et al., 1971). Recently, Shimada et al. (1974) succeeded in...
giving direct evidence of the presence of two different sites in the sugar receptor on chemical modification. Furthermore, Shimada (1975) showed all six amino acids that can stimulate the sugar receptor cell, reacting with the furanose site—one of the two sugar receptor sites. Multiple sites for a single receptor cell may be the molecular basis for the existence of multiple categories of stimulative compounds (e.g., pyranose and furanose sites to sucrose and leucine, respectively). At the level of the receptor molecule (site), the chemoreceptor cells may be regarded as highly specific. Can each site then exactly correspond to each category of compounds?

From the comparison structure of six amino acids with that of monosaccharides in the furanose form essential for stimulation, Shimada (1975) suggested that essential factors for stimulation were a carbon chain with an adequate length, and a carboxyl group and an amino group in the amino acids. In order to examine the rigid stereospecificity of the furanose site for amino acids, we report here the effectiveness of amino acid derivatives and fatty acids on the sugar receptor of the fleshfly.

**MATERIALS AND METHODS**

4–6-day old fleshflies *Boettcherisca peregrina* raised in my laboratory were used in both electrophysiological and behavioral experiments. The adult flies were usually kept on a 3% sucrose solution ad libitum at room temperature just before the electrophysiological experiment, but they were starved for 24 h before the behavioral test.

D-Valine, D-phenylalanine, N-acetyl-L-valine, N-acetyl-L-phenylalanine, and all L-amino acids except L-valine were purchased from Ajinomoto Co., Kawasaki. L-Valine, L-α-amino butyric acid, L-norleucine, L-valine methyl ester, and L-phenylalanine methyl ester were obtained from Nakarai Chemicals Ltd., Kyoto. D-Leucine, d-isoleucine, d-tryptophan, DL-α-aminobutane, glycyl-L-leucine, sodium DL-α-methylbutyric acid, sodium caproate, and L-leucic acid were the products of Tokyo Kasei, Tokyo. Sodium acetate, sodium propionate, sodium butyrate, sodium isovalerate, isovaleric acid, and valeraldehyde were from Wako Pure Chemicals, Osaka. L-Valine amide and N-methyl-L-valine were obtained from Sigma Chemical Co., St. Louis, Mo. L-α-Aminobutanol and L-α-amino butyric acid were purchased from Pfaltz & Bauer Inc., New York. L-Methionine was the product of Nippon Rikagaku Yakuhin, Co., Tokyo.

*Electrophysiology*

The isolated head of a fly was mounted on a piece of platinum wire which served as an indifferent electrode. The responses of chemoreceptor cells were recorded by means of a glass capillary electrode containing Waterhouse's saline (Buck, 1953) from the sidewall of the sensory seta (Morita and Yamashita, 1959). The setae used were of the largest type. Records of the response of the seta of the fleshfly to various chemicals usually show three types of spikes. Spikes from the sugar receptor cell are the largest among those of all receptor cells. The smallest and medium spikes are from the water and salt receptor cells, respectively (Shiraishi and Kuwabara, 1970).

The magnitude of the response was defined as the number of the largest impulses that was counted for 0.2 s starting at 0.1 s after the beginning of the stimulus. The duration of stimulation was less than 0.5 s. The intervals between stimuli were 3 min. The stimulative effectiveness of each chemical was always calculated relative to the control response of the same preparation to the standard chemical, L-valine, one of the most simple amino acids that can stimulate the sugar receptor of the fleshfly (Shiraishi
Stimulation of Sugar Receptor by Fatty Acids, Amino Acid Derivatives

and Kuwabara, 1970). Stimulus solutions were applied to the tip of the seta with another glass capillary. To prevent concentration changes in stimulus solutions by evaporation, the preparation was continuously aerated with air saturated with water vapor, and the stimulus solution in the capillary was renewed before each trial. All stimulus substances were dissolved in redistilled water or in a suitable buffer according to circumstances (see Results). The phosphate buffer was prepared by mixing Na₂HPO₄ and NaH₂PO₄ solutions in the same concentration. We most frequently used a stimulus concentration of 0.01 M, at which the magnitude of the response to most stimulative amino acids reached near maximum but never went to excess. The ambient temperature in the course of the experiment was 22° ± 1°C.

Behavioral Tests
All the flies were starved for 24 h (only water was administered), and were examined for extension of the proboscis to distilled water. Just before application of the stimulus, those flies that responded to water were allowed to drink until the proboscis was retracted. Under these conditions sugars and fatty acids were applied to the prothoracic legs or the labella of the flies. After each test, the prothoracic legs were dipped into water and wiped dry with a piece of filter paper. A minimum of 5 min elapsed between tests of different stimuli on the same fly. The ambient temperature in the behavioral experiments was also 22° ± 1°C. The relative humidity of the experiment room was maintained at 62-80% and did not change more than 5% during any series of experiments.

RESULTS
In order to determine the stereospecificity for amino acids of the sugar receptor, the responses to D-amino acids and the effect of chain length of stimulative L-amino acids were first examined. Next were tested the responses to amino acid derivatives after consideration of the effect of pH on the responses.

Responses to D-Amino Acids
Experiments were performed with six different D-type amino acids which corresponded to L-type amino acids that can stimulate the sugar receptor (Shiraishi and Kuwabara, 1970). D-α-Aminobutyric acid was also examined since L-α-aminobutyric acid was found to stimulate the sugar receptor in this experiment. L-Valine was always examined in each experiment as the standard stimulus. All the chemicals were dissolved in redistilled water, here and in the next experiment. The results are presented in Table I. Relative response means the ratio of the magnitude of the response to each amino acid to that to 0.01 M L-valine. A general conclusion of this study is that D-isomers are markedly less stimulative than the corresponding L-isomers. Slight effectiveness of D-leucine (0.25 ± 0.11) may partially come from contamination with the L-isomer but must be examined in further detail. As far as the results in Table I are concerned, the ineffectiveness of D-amino acids, whose α-amino groups are always the reverse of those of L-isomers, may indicate an important role of the α-amino group in a suitable direction for stimulation by amino acids. It may further indicate that D-amino acids cannot combine with the receptor site since D-amino acids such as 0.01 M D-valine and D-phenylanine mixed in solutions of L-isomers show no inhibitory effect on the responses.
Effect of Chain Length of Amino Acids

The relative response (L-valine = 1) to L-alanine (n = 1, where n is the number of carbon atoms in the side chain) is only 0.03, while the relative responses to L-α-aminobutyric acid (n = 2), L-norvaline (n = 3), L-norleucine (n = 4) are 0.72, 0.88, and 0.82, respectively (Table II). The distinct effect of the length of the side chain can be observed between L-alanine (n = 1) and L-α-aminobutyric acid (n = 2). This is clearly shown from the response-concentration relationship for these two amino acids. The data in Fig. 1 were obtained in one series of experiments. The response to L-α-aminobutyric acid increases as the concentration increases, while that to L-alanine remains at zero up to 0.05 M. Above 0.05 M, the response to L-α-aminobutyric acid has the tendency to decrease. That to L-alanine, on the other hand, often shows the reverse tendency. Thus, a distinct effect of the length of the side chain appears over a wide range of concentrations.

Effect of pH on the Response to Amino Acid Derivatives

In the course of examination of the effectiveness of amino acid derivatives, including fatty acids dissolved in redistilled water, some were found unable to

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### Table I

**Relative Response to D-Amino Acids**

| Chemicals                  | Concentration | Relative response ± SD | No. tests |
|----------------------------|---------------|------------------------|-----------|
| L-Valine                   | 10            | 1.0                    | 101       |
| D-Valine                   | 20            | 0 ± 0                  | 3         |
| L-Phenylalanine            | 10            | 1.08 ± 0.15            | 6         |
| D-Phenylalanine            | 10            | 0 ± 0                  | 4         |
| L-Leucine                  | 10            | 0.92 ± 0.11            | 5         |
| D-Leucine                  | 10            | 0.25 ± 0.11            | 7         |
| L-Isoleucine               | 10            | 0.89 ± 0.21            | 5         |
| D-Isoleucine               | 10            | 0 ± 0                  | 5         |
| L-Tryptophan               | 10            | 0.80 ± 0.24            | 5         |
| D-Tryptophan               | 10            | 0 ± 0                  | 5         |
| L-Methionine               | 10            | 0.72 ± 0.13            | 5         |
| D-Methionine               | 10            | 0.03 ± 0.06            | 5         |
| L-α-Aminobutyric acid      | 10            | 0.72 ± 0.20            | 33        |
| D-α-Aminobutyric acid      | 10            | 0.06 ± 0.08            | 5         |

All chemicals were dissolved in redistilled water.

### Table II

**Effect of Side-Chain Length of Amino Acids**

| Chemicals                  | Concentration | Relative response ± SD | No. tests |
|----------------------------|---------------|------------------------|-----------|
| L-Alanine                  | 10            | 0.03 ± 0.08            | 19        |
| L-α-Aminobutyric acid      | 10            | 0.72 ± 0.20            | 33        |
| L-Norvaline                | 10            | 0.88 ± 0.18            | 10        |
| L-Norleucine               | 10            | 0.82 ± 0.18            | 10        |

All chemicals were dissolved in redistilled water.
stimulate the sugar receptor but, at the same time, found also to inhibit the response of the water receptor that was usually observed in stimulation by seven amino acids that are able to stimulate the sugar receptor cell. Fig. 2 A shows a record of the stimulation of one chemosensory seta by 0.01 M L-valine. It is typical of the records usually obtained in stimulation by the seven amino acids. The largest spikes from the sugar receptor cell, as well as the smallest ones from the water receptor cell, can easily be observed. A few medium spikes appearing in the record may come from the salt receptor cell (cf. Shiraishi and Kuwabara, 1970). Fig. 2 B shows a typical record of the response to 0.01 M L-leucic acid dissolved in water. L-Leucic acid is the derivative obtained by substituting the α-amino group of L-leucine with a hydroxyl group. A few initial spikes were sometimes observed but even the water spikes, as well as the sugar spikes, were almost completely inhibited from 0.1 s after beginning the stimulus. A rebound from the sugar receptor cell was usually observed after removal of the stimulus. Fig. 2 C shows the record of the response to 0.01 M L-leucic acid dissolved in M/15 phosphate buffer (final pH, 6.8). The response was similar to that to 0.01 M L-valine. The largest spikes as well as the smallest ones appeared clearly, which may indicate the possibility that L-leucic acid itself can stimulate the sugar receptor cell, since the phosphate buffer (M/15) itself evokes no response by the sugar receptor but evokes a somewhat lower frequency of water spikes than water does. Phosphate buffer contains several ions, including H⁺, OH⁻, PO₄³⁻, HPO₄²⁻, H₂PO₄⁻, and Na⁺. The difference in the effectiveness between L-leucic acid dissolved in water and that in M/15 phosphate buffer may be explained by the following factors. (a) The pH of the solution of 0.01 M L-leucic acid dissolved in water was 2.9. Shiraishi and Morita (1969) reported the inhibitory effect of low pH ranges below pH 3 on the response to sucrose. Furthermore, these investigators observed that the pH inhibition curve in the acidic pH region shifted toward a higher pH with application of stimuli of lower concentrations. An inhibitory effect of low pH
on the sugar receptor must first be taken into consideration. (b) An excitatory effect of salts (for example, 0.02–0.5 M for NaCl) was observed on the response of the labellar sugar receptor of the flesh fly to sucrose, although the salt itself cannot stimulate the sugar receptor at these concentrations (Morita et al., 1966). M/15 phosphate buffer used at neutral pH contains nearly 0.1 M sodium ions. They might enhance the excitatory effect of L-leucic acid on the sugar receptor at this concentration. (c) An excitatory effect of some other ions, especially the phosphate ion, must be considered at present since their effect on the sugar receptor has not fully been examined yet.

The latter two possibilities, however, may be excluded by the following investigation on the effect of ions on the response to L-leucic acid. A solution of 0.01 M L-leucic acid dissolved in 0.05 M HEPES (one of Good's buffers, pH 7.0) as well as the same concentration dissolved in phosphate buffer evoked the largest impulse discharge. The Good's buffer does not contain any phosphate ion, but contains sodium ion. When dissolved in 0.01 M NaCl solution, L-leucic acid never evoked any impulse discharge in the same manner as in plain water (cf. Fig. 1 B). Besides, when dissolved in 0.1 M imidazole-HCl buffer (pH 7.0) that contained neither phosphate ions nor sodium ions, 0.01 M isovaleric acid could evoke the largest impulse discharge, as will be described later in more detail.
Stimulation of Sugar Receptor by Fatty Acids, Amino Acid Derivatives

...detail. The fatty acid in water was also as ineffective as L-leucic acid. All these results may support the view that the ions in the phosphale buffer at neutral pH cannot play any principal role in the establishment of stimulation and that L-leucic acid is stimulative in itself. Furthermore, each amino acid derivative in water shows diverse pH values according to its dissociation constants. All amino acid derivatives were then dissolved in M/30 phosphate buffer (pH 7.2) in order to make the conditions as uniform as possible.

Responses to Amino Acid Derivatives

The specificity of stimulation by amino acids was studied by testing a number of amino acid derivatives for their effectiveness. The results are shown in Table III. All the chemicals were dissolved in M/30 phosphate buffer (pH 7.2). The concentration of most chemicals was 0.01 M as was mentioned in Materials and Methods.

LENGTH OF SIDE CHAIN The results were almost the same as in Table I. Distinct effects of the side chain can also be seen between L-alanine and L-α-aminobutyric acid. The relative response to L-α-aminobutyric acid seems somewhat smaller than that of Table II. The difference, however, may be ignored because of their large standard deviations.

α-CARBOXYL GROUP Substitution of the α-carboxyl group with —OH (L-α-aminobutyric acid vs. L-α-aminobutanol), esterification of the group (L-valine vs. L-valine methyl ester), or acid amide formation of the group (L-valine vs. L-valine amide) resulted in markedly decreased responses, which may indicate an essential role of the α-carboxyl group in the stimulation by amino acids.

POSITION OF AMINO GROUP Amino acids whose amino group is located at a position other than the alpha, such as DL-β-amino butyric acid and γ-aminobutyric acid, were almost ineffective. This also seems to suggest an important role of the α-amino group, as indicated in Table I.

α-AMINO GROUP Certain modifications of the α-amino group, such as acylation (L-valine vs. N-acetyl-L-valine), methylation (L-leucine vs. N-methyl-L-leucine), and peptide linkage formation (L-leucine vs. glycyl-L-leucine), decreased the response slightly, but the effectiveness of these derivatives was clearly maintained to an appreciable extent. On the other hand, various replacements of the α-amino group with —OH (L-leucine vs. L-leucic acid), —CH₃ (L-α-aminobutyric acid vs. DL-α-methylbutyric acid), —H (L-α-aminobutyric acid vs. butyric acid), or keto function (L-α-aminobutyric acid vs. α-ketobutyric acid), did not show any considerable decrease of the response. Some of such compounds (L-leucic acid and α-ketobutyric acid) were rather more stimulative than the corresponding amino acids. So far as these data are concerned, it may be possible to say that larger groups than the amino group at the α-position show reduced responses, whereas similar- or smaller-sized ones, regardless of their polarity, can show comparable responses at neutral pH.

In contrast, most remarkable was the complete ineffectiveness of these chemicals when they were dissolved in water. This may be due to the low pH (ranging from 2.2 to 3.4) of the solutions except for glycyl-L-leucine (pH 5.9), as mentioned earlier.

At any rate, all these results may further imply that the α-amino group is not
| Characterization | Chemicals | pH* | Relative response ± SD | No. of tests |
|------------------|----------|-----|------------------------|--------------|
| A, Length of side chain | L-alanine | 7.2 | 0.02 ± 0.05 | 8 |
| | L-α-aminobutyric acid | 7.2 | 0.52 ± 0.21 | 17 |
| | L-norvaline | 7.2 | 0.88 ± 0.12 | 7 |
| | L-norleucine | 7.2 | 1.01 ± 0.28 | 7 |
| B, Substitution at the α-carboxyl group | L-α-aminobutyric acid | 7.2 | 0.52 ± 0.21 | 17 |
| (a) —OH | L-α-aminobutanol | 8.1 | 0 ± 0 | 7 |
| | L-α-aminobutyric acid | 7.2 | 0.52 ± 0.21 | 17 |
| | DL-α-aminobutane | 9.9 | 0 ± 0 | 8 |
| (c) esterification | L-valine | 7.2 | 1.00 | 55 |
| | L-valine methyl ester | 7.1 | 0.14 ± 0.16 | 10 |
| (d) acid amide | L-valine | 7.2 | 1.00 | 55 |
| | L-valine amide | 7.1 | 0 ± 0 | 10 |
| C, Position of amino group | L-α-aminobutyric acid | 7.2 | 0.52 ± 0.21 | 17 |
| | DL-β-aminobutyric acid | 7.1 | 0.02 ± 0.04 | 8 |
| | γ-aminobutyric acid | 7.2 | 0 ± 0 | 8 |
| D, Modification of the α-amino group | L-valine | 7.2 | 1.00 | 55 |
| (a) acylation | N-acetyl-L-valine | 6.6 | 0.51 ± 0.17 | 10 |
| | L-leucine | 7.2 | 1.01 ± 0.22 | 9 |
| | N-methyl-L-leucine | 7.2 | 0.68 ± 0.12 | 9 |
| (c) peptide linkage | L-leucine | 7.2 | 1.01 ± 0.22 | 9 |
| | glycyl-L-leucine | 7.1 | 0.56 ± 0.18 | 9 |
| (d) —OH substitution | L-leucine | 7.2 | 1.01 ± 0.22 | 9 |
| | L-leucic acid | 6.6 | 1.11 ± 0.23 | 9 |
| (e) —CH₃ substitution | L-α-aminobutyric acid | 7.2 | 0.52 ± 0.21 | 17 |
| | DL-α-methylbutyric acid | 6.1 | 0.45 ± 0.20 | 8 |
| (f) —H substitution | L-α-aminobutyric acid | 7.2 | 0.52 ± 0.21 | 17 |
| | butyric acid | 6.5 | 0.53 ± 0.08 | 8 |
| (g) keto acid | L-α-aminobutyric acid | 7.2 | 0.52 ± 0.21 | 17 |
| | α-ketobutyric acid | 6.5 | 0.81 ± 0.23 | 8 |

* All chemicals were dissolved in M/30 phosphate buffer (pH 7.2). The concentrations of most chemicals tested was 0.01 M except for racemates and γ-aminobutyric acid which were examined at 0.02 M.
essential for stimulation by amino acids in spite of several suggestions of an important role for the α-amino group, deduced from the fact that either D-amino acids or L-isomers whose amino group is located at a position other than the alpha are almost ineffective. The most obvious example is butyric acid, a fatty acid obtained by replacement of the α-amino group of L-α-aminobutyric acid with only hydrogen atom. The fatty acid was as stimulative as L-α-aminobutyric acid. This result, however, must be examined in further detail, for sodium salts of the fatty acid can stimulate the fifth cell, other than the sugar receptor cell, of the blowfly Phormia regina Meigen (Dethier and Hanson, 1968).

For example, the activity of the fifth cell is greatly enhanced when 1.0 M sodium valerate is the stimulating solution, in comparison to 1.0 M NaCl. Furthermore, the fifth cell gave a spike with an amplitude close to that of the sugar receptor of the blowfly. In order to determine that the fatty acids can really stimulate the sugar receptor of the fleshfly, the stimulating effect of fatty acids should be carefully examined by testing the response-concentration relationships, the stimulating effect of fatty acid derivatives, the effect of treatment with p-chloromercuribenzoate, the behavioral response, and so on. The experiments were usually compared with those of certain amino acids that were already established to stimulate the sugar receptor. First we performed a comparison of spike patterns of the response to fatty acids with the response to amino acids.

Similar Spike Patterns of the Responses to Amino Acids and Fatty Acids

The response of the labellar seta of the fleshfly to various chemicals was examined in one series of experiments (Fig. 3). At least three types of spikes can be clearly distinguished. Spikes from the sugar receptor cell of the fleshfly are the largest among all receptor cells. The smallest and medium spikes are from the water and salt receptor cells, respectively (Shiraishi and Kuwabara, 1970). Fig. 3 A shows the record of the response to 0.5 M NaCl in water. Trains of the medium spikes were a typical response of the salt receptor. A medium spike which can be observed before the beginning of the stimulus comes from the spontaneous activity of the salt receptor. A few spikes before the various stimuli in most records may be used as a standard to identify the spike heights during the stimulus since most spikes of spontaneous activity are the medium ones from the salt receptor. Fig. 3 B shows the record of the stimulation of the water receptor by redistilled water. A few medium spikes—salt spikes—are often observed among the trains of the smallest from the water receptor (cf. Morita et al., 1966). A somewhat lower frequency of the smallest spikes in the record of the response to 0.1 M phosphate buffer (pH 7.0) only (Fig. 3 C) was usually observed compared with the response to water only. Fig. 3 D shows the record of the response to 0.2 M D-glucose in water. The largest sugar spikes are most frequent, although three types of spikes can be observed. The record in Fig. 3 E shows the response to 0.1 M D-fructose in water. The three largest spikes in the record of stimulation by 0.01 M L-valine in water (Fig. 3 F) may come from the overlapping of the sugar spikes with salt spikes. When dissolved in 0.1 M phosphate buffer (pH 7.0), 0.01 M L-valine evoked a somewhat higher frequency of the largest sugar spikes than that in water, but the smallest water
spikes are clearly less frequent (Fig. 3 G). The largest sugar spikes can be easily identified by comparing their height with that of a medium spike of the spontaneous activity before the stimulus. The records of stimulation of the last two chemicals such as 0.01 M isovaleric acid (Fig. 3 H) and 0.01 M sodium isovalerate (Fig. 3 I) dissolved in 0.1 M phosphate buffer (pH 7.0) have impulse discharge patterns very similar to that of 0.01 M L-valine in buffer (Fig. 3 G) although they show considerably lower frequencies. In the last two records the largest spikes can be distinguished from the medium spikes of spontaneous activity and in height are nearly the same as those in the record of the response to L-valine in buffer. Because of the presence of the fifth cell mentioned in the previous section, we should be careful before concluding that the largest spikes in the response to sodium salt of the fatty acid come not from the fifth, but from the sugar receptor cell. The correspondence of the responses to fatty acids and amino acids was examined in more detail so as to exclude the presence of the largest spikes from the fifth cell responses.

RESPONSE TO CONCENTRATION RELATIONSHIPS The excitability of the fifth cell was reported to appear commonly in the 0.1-0.2 M range and at higher concentrations. The threshold, for example, lay near 0.1 M NaCl for most blowfly setae (Dethier and Hanson, 1968). The frequency of the largest spikes of the response to isovaleric acid, on the other hand, increased as the logarithm of the concentration increased over the concentration range of 0.0001-0.01 M (Fig. 4). This tendency seemed largely similar to that with L-valine, but the

![Figure 3. Responses to various chemicals in water or phosphate buffer. A, 0.5 M NaCl in water; B, water only; C, 0.1 M phosphate buffer only (pH 7.0); D, 0.2 M D-glucose in water; E, 0.1 M D-fructose in water; F, 0.01 M L-valine in water; G, 0.01 M L-valine in phosphate buffer; H, 0.01 M isovaleric acid in phosphate buffer; I, 0.01 M sodium isovalerate in phosphate buffer. Length of lines under the records E and I indicates the duration of stimulation.](https://example.com/figure3.png)
former spikes occasionally appeared at a lower concentration than did the latter. Above 0.01 M the frequency of the former spikes sometimes decreased, a tendency different from that of the latter spikes.

RESPONSE TO FATTY ACIDS AND THEIR DERIVATIVES  The similarity of the specificity of stimulation by amino acids to that by fatty acids can also be observed by testing several fatty acid derivatives for effectiveness (Table IV). The effect of chain length on the response to fatty acids was first examined and its results are shown in Table IV A. Sodium salts of fatty acids with various chain lengths were used since, when dissolved in 0.1 M phosphate buffer, saltfree fatty acids were largely as stimulative as the corresponding salts shown in Table IV B (e.g., isovaleric acid and sodium isovalerate). Distinct effects of chain length can also be clearly observed between sodium propionate and sodium butyrate, although sodium propionate was slightly stimulative. A direct comparison was made between fatty acids and amino acids regarding their chain length and effectiveness, and its result is shown in Fig. 5, in which the datum of ineffectiveness of L-glycine was added. Relative responses to L-amino acids and sodium salts of fatty acids show the similar tendency of a distinct increase in response between the chemicals with three and four in total number of carbon atoms. Even the magnitudes of the relative responses agree fairly well with one another at each number of carbon atoms by reference to standard deviation.

The effect of various modifications of the carboxyl group of valeric acid was examined next and its results are shown in Table IV C. Substitution of the group with —OH (amyl alcohol) and —CHO (valeraldehyde), or esterification (methyl valerate), or acid amide formation (valeramide) markedly decreased the response. This agrees also with the results of the modification of the α-carboxyl group of amino acids (Table III).

EFFECT OF PCMB ON THE RESPONSE TO FATTY ACIDS  Shimada et al. (1974) found that the response of the sugar receptor to glucose was markedly depressed after treatment of the receptor with 0.0005 M p-chloromercuriben-
zoate (PCMB), but the response to fructose was not affected. Responses to stimulative amino acids were also hardly affected (Shimada, 1975). As is shown in Fig. 6, treatment of the sugar receptor with 0.001 M PCMB in 0.1 M phosphate buffer (pH 7.0) for 3 min also markedly depressed its subsequent response to 0.2 M D-glucose, but the responses to 0.01 M isovaleric acid and sodium isovalerate as well as L-valine remained clearly less affected. The tendency to spontaneous recovery, similarly observed with isovaleric acid, sodium isovalerate, and L-valine, was hardly detectable with D-glucose. Further studies revealed that a slight effect of PCMB on the response to L-valine was due to individual variations among the flies. The main result, however, was the similar effect of PCMB on the response to L-valine, isovaleric acid, and sodium isovalerate.

**TABLE IV**

| Characterization | Chemicals          | pH*  | Relative response ± SD | No. tests |
|------------------|--------------------|------|------------------------|-----------|
| A, Chain length  | sodium acetate     | 7.0  | 0 ± 0                  | 5         |
|                  | sodium propionate  | 7.0  | 0.20 ± 0.21            | 5         |
|                  | sodium butyrate    | 7.0  | 0.73 ± 0.10            | 5         |
|                  | sodium isovalerate | 7.0  | 0.95 ± 0.18            | 5         |
|                  | sodium caproate    | 7.0  | 0.70 ± 0.18            | 5         |
| B, Acid and salt | L-valine           | 7.0  | 1.00                   | 11        |
|                  | isovaleric acid    | 6.8  | 0.83 ± 0.11            | 5         |
|                  | sodium isovalerate | 7.0  | 0.95 ± 0.18            | 5         |
|                  | valeric acid       | 6.8  | 0.80 ± 0.13            | 6         |
| C, Derivative of valeric acid | valeric acid | 6.8  | 0.80 ± 0.13            | 6         |
|                  | methyl valerate    | 7.0  | 0 ± 0                  | 6         |
|                  | valeramide         | 7.0  | 0.06 ± 0.10            | 6         |
|                  | amyl alcohol       | 7.0  | 0 ± 0                  | 6         |
|                  | valeraldehyde      | 7.0  | 0.17 ± 0.17            | 6         |
|                  | L-norvaline        | 7.0  | 0.93 ± 0.08            | 6         |

* All chemicals were dissolved in 0.1 M phosphate buffer (pH 7.0).

† Concentration of all chemicals tested was 0.01 M.

**Effect of Ions on the Response to Fatty Acids**

The effect of replacement of phosphate and sodium ions by some other ions in the fatty acid solutions was examined. Fig. 7 shows the three records of the responses to 0.01 M L-valine, 0.01 M isovaleric acid, and 0.01 M sodium isovalerate, respectively, dissolved in 0.1 M imidazole-HCl buffer (pH 7.0) that contains neither phosphate ions nor sodium ions in itself. Just as in the phosphate buffer, however, the latter two chemicals—0.01 M isovaleric acid and 0.01 M sodium isovalerate—evoked almost the same impulse discharge patterns as did 0.01 M L-valine—in the imidazole-HCl buffer. The largest sugar spikes can be easily distinguished from the smallest water spikes as well as the medium spikes of spontaneous activity before stimulus. The magnitude of the responses to all the three chemicals alike was somewhat lower than that in phosphate buffer. The similar stimulative effect indicates, however, that neither
sodium ions nor phosphate ions are always indispensable to the establishment of stimulation by fatty acids and supports the view that some fatty acids are intrinsically as stimulative as the corresponding amino acids.

**Behavioral Test**

It has been inferred that behavioral rejection of the long-chain fatty acid salts is related to the activity of the fifth cell, while rejection of the short-chain members is related to the activity of the salt cell, as with sodium chloride (Dethier, 1976). Behavioral tests, therefore, may be one of the most decisive criteria for the validity of our interpretation that the largest spikes evoked by certain fatty acids correspond to the sugar spikes. Namely, if the fatty acids indeed stimulate the sugar receptor, they should elicit a proboscis extension response of the fly.

![Figure 5. Comparison of relative response to several amino acids and sodium salts of fatty acids relative to their chain length. These results are summarized from data in Tables III and IV. The ordinate represents the ratio of the magnitude of the response to each chemical to the response to 0.01 M L-valine. The range of standard deviation is shown by bars associated with each circle.](image)

Flies starved for 24 h were given water until the response to water disappeared. After checking the absence of feeding response to water, 0.1 M phosphate buffer (pH 7.0) was first applied to a pair of prothoracic legs of the flies. No proboscis extension was initiated. After a 5-min interval, the buffer was applied directly to the labellum, but no proboscis extension was observed. Next, 0.01 M isovaleric acid in the phosphate buffer was applied to the legs, and the flies fully extended the proboscis. When the solution was applied to the labellum, the flies also showed even sucking for a few seconds after full extension of the proboscis. A difference between tarsal and labellar stimulation by fatty acid was sometimes observed after several series of experiments. Labellar stimulation always elicited the initiation of proboscis extension even when tarsal stimulation evoked no feeding response. 0.1 M sucrose solution was then applied to the legs and the labellum. Both stimulations always elicited full extension of the proboscis, spreading of the labellar lobes, and sucking. A clear difference between stimulation by sucrose and stimulation by fatty acid was
usually observed in each behavioral test. The flies sucked a fatty acid solution only for a few seconds, and then retracted the proboscis, while they continued to suck a sucrose solution. With all these differences, the flies clearly initiated proboscis extension to the stimulation of 0.01 M isovaleric acid in phosphate buffer.

**DISCUSSION**

The distinct effect of length of the amino acid side chain may support the view proposed in the previous paper (Shimada, 1975) that amino acids reactive with the furanose site must have more than four adjacent carbon atoms, which makes it possible for them to form a conformation similar to that of the furanose ring. As mentioned in Results, the \( \alpha \)-amino group of L-amino acids is not essential for stimulation, but simply common to the limited members of stimulating amino acids. Furthermore, my results indicate that a group larger than the amino group at the \( \alpha \)-position, such as \(-\text{NHCOCH}_3 \) (N-acetyl-L-valine), \(-\text{NHCH}_3 \) (N-methyl-L-leucine), and \(-\text{NHCOCH}_2\text{NH}_2 \) (glycyl-L-leucine), reduced the response but that a similar- or smaller-sized group, such as \(-\text{OH} \) (L-leucic acid), \(-\text{CH}_3 \) (n-L-a-methylbutyric acid), \(-\text{H} \) (butyric acid), and \( \equiv\text{O} \) (\( \alpha \)-keto butyric acid), regardless of polarity, can show comparable responses at neutral pH. If the negative contribution of the larger group is regarded for the most part as coming from steric hindrance, there may be limited space in the furanose site, hindering a larger group than the amino group at the \( \alpha \)-position, but accepting a similar- or smaller-sized one. The special barrier may be located in the direction opposite to the \( \alpha \)-amino group of L-amino acids, since \( \delta \)-amino acids corresponding to stimulating L-amino acids cannot stimulate the sugar receptor and do not combine with the furanose site. Other barriers at different positions may also be suggested because of the ineffectiveness of \( \beta \)-and \( \gamma \)-aminobutyric acid. We cannot ignore the possibility, however, of an
inhibitory effect of polar or charged groups in the amino acid side chain, as mentioned in an earlier paper (Shimada, 1975). Owing to steric hindrance by the above-mentioned special barriers in the furanose site, the sugar receptor cells may be able to discriminate at least optical isomers, the number of carbon atoms, the position of amino group and so on. On the whole, at the level of the receptor site they may be regarded as highly specific, although the α-amino group is now revealed as dispensable for the establishment of stimulation by amino acids.

![Graph](image)

Figure 7. Records of the responses of L-valine, isovaleric acid, and sodium isovalerate in 0.1 M imidazole-HCl buffer (pH 7.0). A, 0.01 M L-valine; B, 0.01 M isovaleric acid; C, 0.01 M sodium isovalerate. Length of lines under record C indicates the duration of stimulation.

Special attention must be paid to the effect of pH in order to assess the stimulating effectiveness of ionizable compounds such as amino acids, fatty acids, etc., although independence of pH was confirmed in all receptors of the flies over the wide pH range of 3.0–10.0. The absence of a pH effect, however, often varies according to the kind and concentration of stimuli, or the presence of some other ions (Gillary, 1966; Shiraishi and Morita, 1969; McCutchan, 1969). Furthermore, acid solutions contain in addition to hydrogen ions, one or more species of anions, undissociated acid, undissociated water and hydroxyl ions. We must then determine which components are effective and how much each contributes to the net result. Each component of the solutions cannot be
examined separately by merely being dissolved in water. At neutral pH, however, we can ignore hydrogen and hydroxyl ions, as well as undissociated acid because of their low concentration, and we can chiefly evaluate the effectiveness of dissociated anions when the buffer used is ineffective, or when the effect on the receptors, if it exists at all, is examined accurately in advance. According to my results, anions of certain amino acid derivatives and fatty acids are proved to stimulate the sugar receptor only at neutral pH in phosphate buffer. When dissolved in water, the solutions are able not only to stimulate any receptor but also to depress the activities of the water receptor because of their low pH (nearly 3). In general, achieving pH changes by changes in concentration of the acids should be avoided, because changes in concentration of the acids are often made in the study of acid stimulation but result in simultaneous changes of many components in the solution. Certain fatty acids as well as their sodium salts at neutral pH were proved to stimulate the sugar receptor cell by the already-mentioned observations of the similarities to l-amino acids, which may be summarized as follows.

(a) A solution of 0.01 M isovaleric acid and sodium isovalerate, as well as l-valine, evoked the same height of impulses—the largest spikes—as various sugars such as D-glucose, D-fructose, etc., did. (b) Isovaleric acid showed a clear concentration-response relationship over the concentration range of 0.0001-0.01 M which is similar to that of l-valine. (c) The respective magnitudes of the relative responses to sodium salts of fatty acids and the corresponding l-amino acids in the total number of carbon atoms agree fairly well with one another and show the similar tendency of a distinct increase between the chemicals with C₃ and C₄. (d) Various modifications of the carboxyl group of fatty acids as well as l-amino acids markedly decreased the response. (e) The similar effect of PCMB treatment of the receptor on the response to isovaleric acid and its sodium salt to the response to l-valine was observed, in contrast to the response to 0.2 M D-glucose. (f) The water-satiated flies clearly initiated proboscis extension to 0.01 M isovaleric acid in phosphate buffer (pH 7.0) as well as 0.1 M sucrose solution, but never initiated such an extension to phosphate buffer only.

On the other hand, Dethier and Hanson (1968) reported that the sodium salts of fatty acids can stimulate the fifth cell, other than the sugar receptor cell, of the blowfly Phormia regina Meigen. Where does this difference come from? First, it may result from a species difference between the blowfly and the fleshfly, which may also partially explain the difference in the responses between the two species to l-alanine and l-valine (cf. Goldrich, 1973). The former responds preferentially to l-alanine but scarcely at all to l-valine, while the latter shows the reverse reaction, according to my results (cf. Figs. 1 and 3). The second reason may be the difference in the concentration of stimuli used in the experiments. Dethier and Hanson did not test fatty acids at concentrations lower than 0.05 M. They observed that the second salt spike, the excitability of the fifth cell, appeared commonly in the 0.1-0.2 M range and at higher concentrations. For example, the activity of the fifth cell was greatly enhanced when 1.0 M sodium valerate was the stimulating solution, compared with 1.0 M NaCl. Owing to the low frequency of response of the fifth cell to salts, they doubted whether the salt was actually an adequate stimulus. On the other
hand, we observed a clear stimulating effect of certain fatty acid salts at concentrations lower than 0.01 M and a remarkable correlation between the frequency of response and the concentration of stimulus. High concentrations of chemicals, furthermore, may often affect the receptors rather nonspecifically, that is, with the least response-concentration relationship, and cause some injurious effect to them if applied for a fairly long time. In order to determine an adequate stimulus, the lowest possible concentration of the chemicals should be carefully examined, considering all possible factors relating to their effectiveness such as pH effect, concentration dependency, receptor specificity, and so on. The same is true in the behavioral tests.

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