Gustatory Responses of Eel Palatine Receptors to Amino Acids and Carboxylic Acids

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Abstract The gustatory receptors of the eel palate were found to be extremely sensitive to amino acids and carboxylic acids. The results obtained are as follows: (a) 11 amino acids which are among naturally occurring amino acids elicited responses in the palatine nerve, but 9 amino acids did not elicit a response even at a high concentration. The effect of D-amino acids was always much less than that of their corresponding L-isomers. There was no appreciable difference in the effectiveness of an α-amino acid (α-alanine) and β-amino acid (β-alanine). (b) The threshold concentrations of the most potent amino acids (arginine, glycine) were between $10^{-8}$ and $10^{-9}$M. A linear relation between the magnitude of the response and log stimulus concentration held for a wide concentration range for all the amino acids examined. (c) The palatine receptors responded sensitively to various carboxylic acid solutions whose pH was adjusted to neutral. The threshold concentrations varied between $10^{-4}$ and $10^{-7}$M. The magnitude of the response at $10^{-2}$M increased with an increase of carbon chain length. (d) The extent of cross-adaptation was examined with various combinations of amino acids. A variety of the response patterns showing complete cross-adaptation, no cross-adaptation, or synergetic interaction was observed. The synergetic interaction was also observed when one amino acid below its threshold concentration was added to the other amino acid. No cross-adaptation was observed between amino acids and fatty acids. (e) The treatment of the palate with papain led to loss of the responses to arginine, glycine, and histidine without affecting those to proline and acetic acid. The treatment with pronase E eliminated selectively the response to proline. The possibility that the eel gustatory receptors are responsible for sensing food at a distance was discussed.

Introduction

Amino acids are potent chemical stimuli which stimulate chemoreceptors of various organisms from bacteria to higher vertebrates. Gustatory receptors of the terrestrial vertebrates respond to various amino acids and exhibit characteristic responses. For example certain salts of amino acids such as monosodium glutamate have unique flavor-enhancing activity and exhibit synergetic interaction with 5'-ribonucleotides (Sato et al., 1967). The concentration-response

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relation of the gustatory response of the rat to glycine satisfied the Hill
equation with a coefficient of 4.4 (Tateda, 1967), while the coefficient of
responses to most other stimuli is unity (Beidler, 1971). It is not certain
whether amino acids stimulate any of the receptors for the fundamental taste
qualities or whether there exist specific receptors for particular species of
amino acids. Thus there remain many unsolved problems of receptor mecha-
nisms for amino acids.

The responses to amino acids are particularly important for certain species
of fish since they evoke a feeding response. Electrophysiological studies have
revealed that both olfactory and taste receptors are responsive to amino acids
which are usually the most potent stimuli for these receptors (Bardach and
Atema, 1971; Suzuki and Tucker, 1971; Sutterlin and Sutterlin, 1971; Hara,
1973; Kiyohara et al., 1975; Caprio, 1975, 1977; Caprio and Tucker, 1977).
Thus the chemical senses of the fish are a most suitable system to use to
explore the receptor mechanisms for amino acids.

The eel is nocturnal and its chemical senses are considered to be highly
developed. In this study, we find that the gustatory receptors on the palate of
the eel are highly sensitive to amino acids and carboxylic acids. We have
measured the responses to these stimuli by recording palatine nerve activity
under various conditions. Sensitivity, range of the responses, concentration-
response relationships, structure-activity relationships, synergetic interactions
among amino acids, and characteristics of receptor sites are reported in this
paper.

MATERIALS AND METHODS

Measurements of the Palatine Nerve Activity

An eel, Anguilla japonica, ~130–180 g, which was cultured in ponds, was obtained from
a local fishery and used within 4 d. The use of freshly obtained fish resulted in
electrical recordings which lasted up to 24 h. An increase in laboratory holding time
of fish led to a slow decline of the gustatory receptor sensitivity. However, the rate of
decline was not as remarkable as the reported decline in chemoreceptor sensitivity in
the catfish (Caprio, 1975).

A fish was immobilized with gallamine triethiodide (0.01 mg/100 g body wt). After
cutting the lower jaw off, the palatine nerve was exposed. The epithelium sheet at the
back of the palate was torn, and its edge was lifted to make a barrier which prevented
the leakage of the stimulating solution to the nerve area (see Fig. 1). The palatine
nerve was placed on Ag-AgCl bipolar electrodes; mineral oil was added to prevent
drying. Electrical activity from receptors was amplified with a CR-amplifier (capacity
coupled, Nihon Koden Kogyo), passed through an integrator (Nihon Koden Kogyo)
whose time constant was 0.3 s, and displayed on a pen recorder (Nihon Koden
Kogyo).

The chemical stimuli, analytical grade, were dissolved in artificial pond water
(abbreviated as APW, 0.3 mM NaCl, 0.02 mM KCl, and 0.2 mM CaCl₂, pH about
5.7). APW was aerated through a water filter which equilibrated the CO₂ dissolved
in APW with that in air; the palatine receptors of the eel are sensitive to CO₂. Final
pH of solutions of neutral chemicals thus prepared was ~6. The pH of the solutions
of acidic amino acids, carboxylic acids, and basic amino acids was adjusted to ~6
with NaOH or HCl. Freshly prepared amino acid solutions were used. Care was taken to minimize possible sources of contamination such as fingerprints on the glassware.

The palatine receptors are sensitive to touch. The response to touch is phasic and easily adapted by continuous irrigation with APW. The palate was always irrigated with APW. This APW was the same as that in which chemical stimuli were dissolved.

5 ml of a stimulating solution was introduced into the funnel (see Fig. 1) by a pasteur pipette; after stimulation the palate was rinsed with APW. The flow rate of APW or a stimulating solution was 0.25 ml/s at which rate no touch artifacts were observed. In most experiments the interval between stimulus solutions was 4 min for lower concentrations and about 10 min for higher concentrations. The magnitude of the response to each stimulant was always calculated relative to a response to a standard solution of 10^{-5}M glycine of the same preparation. All the experiments were carried out at 21 ± 1°C.
Chemicals

The amino acids and amino acid derivatives used are listed in Table I, in which abbreviations are noted as well as supplier. Formic acid, acetic acid, and butyric acid were purchased from Wako Pure Chemical Industries; valeric acid and caproic acid were purchased from Nakarai Chemical Ltd.; propionic acid and papain were obtained from Merck AG (Darmstadt, West Germany); and pronase E was obtained from Kaken Kagaku Kogyo (Tokyo).

Table I

| Chemicals     | Abbreviation | $10^{-4}$M | $10^{-2}$M | Chemicals     | Abbreviation | $10^{-4}$M | $10^{-2}$M |
|---------------|--------------|------------|------------|---------------|--------------|------------|------------|
| L-Arginine    | Arg          | 127        | 182 *      | L-Valine      | Val          | 0          | 0          |
| Glycine       | Gly          | 125        | 170        | L-Cystine     | Lys          | 24         | 87 *       |
| L-Alanine     | Ala          | 89         | 160        | L-Norvaline   | Pro          | 77         | 144        |
| L-Proline     | Pro          | 56         | 94         | L-Norleucine  | Lys          | 53         | 89 §       |
| L-Lysine      | Lys          | 71         | 143        | L-Aspartic acid | Ser         | 0          | 0          |
| L-Serine      | Ser          | 56         | 94         | L-Glutamic acid | Abu         | 32         | 105        |
| L-$\alpha$-Amino-butyric acid | Abu | 53       | 89 §       | L-Asargine    | L-Histidine  | 24         | 87 *       |
| L-Cysteine    | Cys          | 0          | 92         | L-Glutamine   | Cys          | 0          | 65 §       |
| L-Citrulline  | Cys          | 0          | 53         | L-$\beta$-Alanine | Cys        | 0          | 63 §       |
| L-Threonine   | Thr          | 0          | 53         | $\gamma$-Aminobutyric acid | Thr | 0          | 0          |
| L-Homoserine  | Ile          | 0          | 38         | D-Alanine     | Phe          | 0          | 39         |
| L-Isoleucine  | Ile          | 0          | 0          | D-Arginine    | Ile          | 0          | 0          |
| L-Leucine     | Leu          | 0          | 0          | D-Serine      | Leu          | 0          | 0          |
| L-Phenylalanine | Phe       | 0          | 0          | Glycine methyl-ester | Met | 0          | 34         |
| L-Methionine  | Met          | 0          | 0          | L-Alanine methylester | Met | 0          | 127 §      |
| L-Tryptophan  | Trp          | 0          | 0          | Betanine      | Trp          | 0          | 0          |
| L-Tyrosine    | Tyr          | 0          | 0          | Taurine       | Tyr          | 0          | 0          |

The values are relative responses; the response to the compound tested was divided by the response to $10^{-4}$M Gly × 100. The values reported are averages of two or more determinations.

Chemicals were purchased from *Wako Pure Chemicals Industries, Osaka, *Kyowa Hakko Kogyo Co., Tokyo, §Nakarai Chemicals Ltd., Kyoto, or Fluka AG., Basel, Switzerland.

RESULTS

Responses to Amino Acids

Fig. 2 illustrates the summated responses to glycine of varying concentrations. Responses to lower concentrations are of a tonic type but phasic components appear prior to tonic components at high concentrations. The response ends quickly when APW is applied to the palate.

Fig. 3 A, B, and C illustrate concentration-response relationships for various amino acids and betaine. What is plotted (R) is the peak height of the
summated response divided by the response to $10^{-8}$M glycine $\times 100$. The relationship between the response and log stimulus concentration is linear for the wide concentration range of all the amino acids examined. The straight lines in the figures are fit by least squares method without regard to the zero response points. The lines are extended, on the graph, through the zero response level and the concentrations indicated at the crossing are referred to as the thresholds. The thresholds thus determined vary for different amino acids and are between $10^{-9}$ and $10^{-3}$M for glycine (Gly) and L-arginine (Arg), between $10^{-8}$ and $10^{-7}$M for L-alanine (Ala), between $10^{-7}$ and $10^{-6}$M for L-proline (Pro), L-lysine (Lys), L-serine (Ser), and L-$\alpha$-aminobutyric acid (Abu) and between $10^{-5}$ and $10^{-4}$M for L-histidine (His) and betaine.

Table I shows a comparison of the stimulatory effectiveness of 32 amino acids and their derivatives tested at $10^{-4}$ and $10^{-2}$M. Among naturally occurring amino acids, only seven amino acids (Arg, Gly, Ala, Pro, Lys, Ser, His) were effective at $10^{-4}$M, nine (Ile, Leu, Phe, Met, Tyr, Trp, Val, Asp, Glu) did not elicit any response even at $10^{-2}$M. None of the three D-amino acids tested were as effective as their corresponding L-isomers. There was no appreciable difference in the effectiveness between $\alpha$ and $\beta$ L-alanine, but $\gamma$-aminobutyric acid was much less effective than $\alpha$-aminobutyric acid. Replacement of carboxylic group with sulfonate ($\beta$Ala$\rightarrow$taurine) led to complete loss of the activity. The effectiveness of L-$\alpha$-amino acids with hydrocarbon chains was decreased with an increase in length of the carbon chain (Gly$>$Ala$>$Abu) and the amino acids having more than five carbon atoms (Val, Leu, Ile, norvaline, norleucine) elicited no response. Acidic amino acids (Glu, Asp) elicited no response but acid amides of these amino acids, L-glutamine and L-asparagine, were effective at $10^{-2}$M. Aromatic amino acids (Phe, Trp, Tyr) elicited no response. Methyl esterification of glycine and L-alanine produced an appreciable decrease in the activity, but these esters still exhibited considerably high activity at $10^{-2}$M.

![Figure 2. Typical summated taste responses from the eel palatine receptors when stimulated with glycine at eight different concentrations.](image-url)


Response to Carboxylic Acids

The palatine receptors were also sensitive to carboxylic acids. Fig. 4 illustrates a typical concentration series for propionic acid, adjusted to pH 6 with NaOH. The response appears at $10^{-6}$M and increases with concentration. The response at $10^{-6}$M is of tonic type and a phasic component appears at $10^{-5}$M. The tonic component is considerably increased at $10^{-4}$M. Such a large increase in the tonic component was also observed with caproic acid and valeric acid above $10^{-3}$M and with butyric acid above $10^{-2}$M. Carboxylic acid solutions adjusted with NaOH to pH 6 contain sodium ions, however, the sodium ion probably is not itself a significant stimulus since $10^{-2}$M NaCl evokes only a small response.

As shown in Fig. 5, plots of the magnitude of the response to acids (or their

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**Figure 3** Relative magnitude of the responses ($R$) to amino acids and betaine as a function of log stimulus concentration (log C). The peak height of the summated response was taken as the magnitude of the response. Responses plotted ($R$) were calculated relative to the response to $10^{-4}$M glycine and multiplied by 100. In (A) glycine, L-alanine and L-α-aminobutyric acid; in (B) L-arginine, L-lysine, and L-histidine; and in (C) L-proline, L-serine, and betaine relative responses are plotted. The straight lines were fit by the least squares method without regard to the zero response point. Correlation coefficient of the data for Lys was 0.983 and those for other amino acids and betaine were more than 0.995.
salts), except for butyric acid, against stimulus concentration do not give a straight line, which contrasts with such plots for amino acids. Dotted lines are the extension of the curves to the zero response level, and the concentrations where the dotted lines cross the level are referred to as thresholds. The threshold concentration has its lowest value at three carbon atoms. On the other hand, the magnitude of the responses to carboxylic acids at $10^{-2}$M increases with an increase in length of the carbon chain of the acids. Both relationships described above are quite different from that observed with unbranched aliphatic amino acids.

**Effect of pH and Ions on the Responses**

There was no significant difference in the evoked response to $10^{-8}$M glycine at pH 5.0 and 9.0. The response to $10^{-4}$M propionic acid at pH 9.0 was a little smaller than that at pH 5.0. The responses to glycine and propionic acid below pH 5.0 or above pH 9.0 were not examined since the responses to the added HCl or NaOH themselves appeared in these pH regions.
In order to examine the effect of the elimination of ions from the palatine receptors, the palate was irrigated with distilled water instead of APW for 30 min, before $10^{-6}$M glycine dissolved in distilled water was applied. This treatment, which served to eliminate ions, did not bring about any appreciable change in the response to glycine. Treatment of the palate with 10 mM glycoletherdiamine $N,N,N',N''$-tetracetic acid (EGTA) for 10 min also had no significant effect on the response to glycine. Furthermore, addition of 10 mM NaCl, 10 mM KCl, and 10 mM CaCl$_2$ to the solutions of glycine also did not affect the response. Similar results on the elimination and addition of ions were also obtained with other amino acids and carboxylic acids as stimuli.

**Cross-Adaptation**

In order to understand how the eel palatine receptors may discriminate various amino acids and carboxylic acids, the extent of cross-adaptation between these stimuli was examined. A method similar to that of Smith and Frank (1972) who examined the extent of cross-adaptation between salts in the rat chorda tympani nerve was employed. The experiments were carried out as follows. For example, $10^{-5}$M glycine was applied first to the palate and was followed by $10^{-5}$M L-arginine after the response to glycine had declined. Then the order of application of the stimuli was reversed; arginine was applied first and subsequently glycine was applied. The concentrations of two stimuli were chosen so that approximately equal magnitudes of the response were evoked when they were applied alone. The response patterns for combinations of various stimuli were roughly classified into three types: I, II, and III. Type I shows no appreciable second peak. Type II shows a distinct second peak whose magnitude is practically unaffected by application of the first stimulus. In type III, the second response is greatly enhanced by application of the first stimulus. There were intermediate types especially between types I and II.

Fig. 6 A shows typical patterns of type I. L-Arginine ($10^{-5}$M) after glycine ($10^{-5}$M) did not elicit any appreciable peak response; glycine after L-arginine also elicited no second peak. Fig. 6 B shows typical patterns of type II. L-Proline ($10^{-5}$M) after L-arginine ($10^{-5}$M) elicited a distinct second response. The magnitude of the second response was practically unchanged by the previous application of L-arginine (compare peaks 2 and 3). L-Arginine after L-proline also elicited a distinct response comparable to that to L-arginine applied as the first stimulus (compare peaks 1 and 4). Fig. 6 C shows a typical pattern of type III. L-Histidine ($10^{-5}$M) after L-arginine ($10^{-5}$M) elicited a remarkably large response even though the control response to L-histidine (peak 3) was rather small. The response to L-arginine after L-histidine was also greatly enhanced.

Table II represents types of the response patterns obtained with various combinations of stimuli. Seven naturally occurring amino acids which are effective at $10^{-4}$M (see Table I) are used for the combinations. Type I is obtained with combinations among glycine, L-alanine, L-arginine, and L-lysine. Combinations of L-proline or betaine with other stimulants give either type II or type III. Combinations of L-histidine with other stimulants except for glycine give type III.
The extent of cross-adaptation between amino acids and carboxylic acids which have the same carbon chain length was also examined. Combinations of acetic acid and glycine and of propionic acid and L-alanine, and the combination of butyric acid and L-α-aminobutyric acid gave response patterns of type II.

**Synergetic Effect**

The results of the cross-adaptation experiments suggested that a synergetic interaction exists between certain species of amino acids. This synergetic interaction was examined by adding one amino acid, L-histidine, of varying concentrations below the threshold to other amino acids of a fixed concentration.

Fig. 7 A shows typical responses to the mixture of $10^{-8}$M L-arginine and L-histidine of varying concentrations equal to or below $10^{-5}$M. The figure shows that L-histidine increases the responses although it gives no response itself in the concentration range employed. The magnitude of the response to the mixture of $10^{-8}$M L-arginine and $10^{-5}$M L-histidine was approximately twice that to $10^{-8}$M L-arginine alone. Fig. 7 B shows responses to the mixture of
$10^{-2}\text{M}$ betaine and $L$-histidine of varying concentrations equal to or below $10^{-5}\text{M}$. In this case, the magnitude of the peak height for the mixture is not enhanced much but the magnitude of the tonic response is enhanced. A similar enhancement of the tonic response was also observed with mixture of $10^{-3}\text{M}$ $L$-proline and $L$-histidine of varying concentrations.

**Treatment of the Palate with Proteases**

Fig. 8 A (left) illustrates the summated responses to $10^{-5}\text{M}$ glycine, $10^{-6}\text{M}$ $L$-arginine, $10^{-4}\text{M}$ $L$-proline, $10^{-2}\text{M}$ $L$-histidine, and $10^{-2}\text{M}$ acetic acid before and after the palate is treated with 5% papain solution for 30 min. The

| Gly | Ala | Lys | Arg | Ser | Pro | His |
|-----|-----|-----|-----|-----|-----|-----|
| type | log C | type | log C | type | log C | type | log C | type | log C | type | log C |
| Ala | I | -5 | | | | | | | | | | |
| | | | | | | | | | | | | |
| Lys | I | -5 | I | -3 | | | | | | | | |
| | | | | | | | | | | | | |
| Arg | I | -5 | | | | | | | | | | |
| | | | | | | | | | | | | |
| Ser | I | -5 | | | | | | | | | | |
| | | | | | | | | | | | | |
| Pro | II | -5 | | | | | | | | | | |
| | | | | | | | | | | | | |
| His | II | -5 | | | | | | | | | | |
| | | | | | | | | | | | | |
| Betaine | II | -5 | | | | | | | | | | |

Types I, II, and III are represented by I, II, and III. The concentrations of stimuli used in the cross-adaptation experiments are presented in the column for log C where the upper and lower values represent logarithmic concentrations of respective stimuli indicated above and to the left, respectively.

The use of papain or pronase E solutions which were dialized thoroughly against APW gave the same results, and hence, it is unlikely that substances of low molecular weight which contaminate the proteases contrib-
ute to the suppression of the responses. It is probable that the receptor proteins for amino acids are eliminated from the surface of the receptor membranes by the proteolytic action of the proteases, or that the proteases suppress the responses to amino acids by adsorbing strongly on the receptor sites for amino acids.

Figure 7. Typical summated responses to the mixture of amino acids and betaine where one stimulus of varying concentrations below threshold was added to a stimulus of a fixed concentration. (A) The responses to the mixture of 10^{-6}M Arg and His of varying concentrations equal to or below 10^{-8}M; (B) the responses to the mixture of 10^{-6}M betaine and His of varying concentrations equal to or below 10^{-8}M.

The above results indicate that the receptors for L-arginine, glycine, and L-histidine are different from that for L-proline or carboxylic acids. This is consistent with the results shown by Fig. 6 where the extent of cross-adaptation between these stimuli was examined. The above results do not imply that L-arginine, glycine, and L-histidine stimulate the same receptor site.
DISCUSSION

Hashimoto et al. (1968) and Konosu et al. (1968) examined the effect of the extracts of short-necked clam on the exploratory and feeding behavior in eels and reported that the stimulating activity of the extracts is attributable to amino acids. Among 18 amino acids tested, 3 amino acids (Arg, Ala, Gly) were the most effective when tested individually. However, the mixture of amino acids had a more potent effect than individual amino acids, and hence they suggested that the stimulating effect of the extracts is attributable to a synergetic or additive interaction between amino acids. In their experiments, the test sample was put in a place distant from the compartment where the eels were swimming, and the number of eels gathering near the test sample was counted. Hence, the results obtained by Hashimoto et al. (1968) suggested that the “distance sense” of the eels is sensitive to a mixture of amino acids.

The results obtained in the present study indicated that the gustatory receptors of the palate of the eel are extremely sensitive to amino acids, especially arginine, glycine, and alanine, and that there exists synergetic interactions between histidine and other amino acids. Thus, the present results are closely correlated with the observations of exploratory and feeding behavior. These facts can be explained by the following two ways. Either the sensitivity and specificity of the olfactory receptors to amino acids are quite similar to those of the gustatory receptors or the gustatory sense is responsible for sensing food at a distance. The acuity of the gustatory receptors to amino acids presented in this paper suggests the latter possibility, although the olfactory receptors may also contribute to sense food at a distance. A similar comment was made by Caprio (1975, 1977) who reported that the taste receptors on the maxillary barbel of the catfish are extremely sensitive to amino acids.

It is known that the chemoreceptors of various organisms respond to amino acids. These chemoreceptors, e.g., gustatory receptors of the catfish (Caprio, 1975, 1977), olfactory receptors of trout and salmon (Hara, 1973; Sutterlin and Sutterlin, 1971), or contact chemoreceptors of the fleshfly (Shiraishi and Kuwabara, 1970; Shimada, 1978), respond more or less to most of naturally occurring amino acids. On the other hand, the palate gustatory receptors of the eels respond only to 11 amino acids; 9 amino acids did not elicit any response even at high concentrations. Such strict specificity contrasts to the specificity in the chemoreceptors of other organisms.

The present results show that a linear relation between the magnitude of the response to amino acids (R) and log stimulus concentrations (log C) held for a wide range of stimulus concentration varying from 3 to 7 for all the amino acids examined. In the case of glycine and L-arginine, the approximately linear relation held over about seven log units. On the other hand, the gustatory and olfactory receptors of the catfish show a linear relation between log R and log C in a wide range of stimulus concentration (Caprio, 1975, 1977).

A linear relation between R and log C in a wide concentration range cannot be explained simply by the Beidler's taste equation; the linear relation between R and log C holds only within two log units for the taste equation or the Langmuir isotherm. A theory to explain the linear relation in a wide concen-
The concentration range is not yet established, but the relation may be explained by the following two ways. Either one species of amino acid binds to several receptor sites with different binding constants, or a negative cooperativity exists between receptor sites; the binding of a stimulant to a receptor site decreases the affinity of the surrounding sites to the stimulant. A synergetic interaction between certain pairs of amino acids may be brought about by weakening of negative cooperativity or by positive cooperativity between certain receptor sites.

The explanation of the results on the effect of cross-adaptation is not simple since the mechanism of adaptation of taste responses is not known. It has been proposed (Beidler, 1953, 1962; Hellekant, 1969) that adaptation results when receptor sites are filled by the taste stimulus. Kamo et al. (1979) postulated that the transformation of an active receptor-stimulus complex into an inactive complex is responsible for the adaptation. According to this idea, the extent of cross-adaptation between two stimuli indicates the extent to which they compete for the same receptor sites. For the pairs of stimuli which elicit type II or type III patterns, the response to the second stimulus was not suppressed by the first stimulus; on this basis, it would be said that the pair of stimuli stimulate different receptors. The results obtained with use of the proteases indicated more directly that the receptor protein for L-proline is different from that for other amino acids. On the other hand, it may not be simply concluded that a pair of stimuli which shows a type I pattern compete for the same receptor site. Smith and Frank (1972) pointed out that receptor sites may be relatively nonspecific or that prolonged stimulation may render taste receptor cells less excitable; if this were so, adaptation to one stimulus would prevent the receptor from responding to others which fill sites on the same cells. For example, the present study shows that a pair of arginine and alanine gives type I pattern, but it may not be simply concluded that both stimuli stimulate the same receptor. In fact, Caprio and Tucker (1977) stated that alanine and arginine stimulate independent receptors on the maxillary barbel of the catfish, based on the cross-adaptation experiment and single fiber analysis.

A synergetic interaction was observed with certain pairs of amino acids. The experiments shown by Fig. 7 were carried out under the condition where concentration of one stimulus was varied below the threshold concentration

FIGURE 8. (A–left panel) Effect of the papain treatment on the summated responses to amino acids and acetic acid. The palate was treated with 5% papain dissolved in APW containing \(10^{-3}\)M Tris-HCl buffer of pH 7.4 for 30 min. After the palate was washed with APW, \(10^{-3}\)M Gly, \(10^{-4}\)M Arg, \(10^{-5}\)M Pro, \(10^{-5}\)M His, and \(10^{-7}\)M acetic acid were applied. The interstimulation interval was about 1 min. (a) Before treatment; (b) immediately after treatment; (c) 1 h after treatment. (B–right panel) Effect of the pronase E treatment on the summated responses to amino acids and acetic acid. The palate was treated with 5% pronase E dissolved in APW containing \(10^{-3}\)M Tris-HCl buffer of pH 7.4 for 30 min. After the palate was washed with APW, \(10^{-4}\)M Gly, \(10^{-4}\)M Arg, \(10^{-5}\)M Pro, \(10^{-5}\)M His, and \(10^{-7}\)M acetic acid were applied. The interstimulation interval was about 1 min. (a) Before treatment; (b) immediately after treatment; (c) 20 min after treatment.
and hence the enhancement observed in Fig. 7 is purely due to the synergetic interaction. The synergetic interaction is greater if the first stimulus in a cross-adaptation is above threshold concentration (see Fig. 6). When two amino acids, both above threshold were mixed, greater enhancement was also observed.

The palatine receptors of the eel respond well to carboxylic acids. The results shown by cross-adaptation suggest that these acids and amino acids stimulate independent receptors. This was confirmed by the treatment of the palate with papain which led to loss of the response to amino acids except for proline without affecting the responses to carboxylic acids. This contrasts with the results of Shimada (1978) who found that the acids stimulate the same receptors as amino acids in the fleshfly. Since carboxylic acids elicit a large response even at pH 9.0, the anions of the acids seem to contribute to the responses. Beidler (1954) showed that sodium salts of carboxylic acids are less effective in producing responses of the rat gustatory receptors than NaCl, and that an increase of the carbon chain length led to decrease of the response. The eel palatine receptors are much more sensitive to carboxylic acids than the receptors of the rat, and this order of effectiveness is quite different from that in the rat; the response of the palatine receptors to carboxylic acids increase with increasing carbon chain length. The present results resemble those of Sutterlin and Sutterlin (1970) who observed that the taste responses of the Atlantic salmon to the acids increase with increasing carbon chain length. It seems that the carboxylic acids do not stimulate the salt receptors in the eel palate but stimulate the receptors responsive to carboxylic anions.

The elimination of ions to the eel palate did not bring about any significant effect on the responses to amino acids and carboxylic acids. This suggests that the permeability change of ions across the receptor membrane does not contribute to the generation of the response which is similar to the case for the rat gustatory receptors (Beidler, 1967).

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