High Sensitivity of Minnow Gustatory Receptors to Amino Acids

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KIYOHARA, S., S. YAMASHITA AND S. HARADA. High sensitivity of minnow gustatory receptors to amino acids. PHYSIOL. BEHAV. 26(6) 1103-1108, 1981.—The stimulating effects of amino acids and related compounds on the gustatory receptors were studied in the Japanese minnow, Pseudorasbora parva, by recording electrical responses from the palatine nerve innervating the upper lip and the adjacent palate. All of the 21 amino acids and 6 related compounds elicited responses at a concentration of $10^{-3} \text{ M}$. The order of the response magnitude to the 6 most effective of 18 L-amino acids was: proline > lysine-HCl > alanine > arginine-HCl > cysteine > serine. The threshold concentration for proline, the most potent among the amino acids was estimated to range between $10^{-1.5}$ and $10^{-0.8} \text{ M}$. The relationship between the log response magnitude and the log stimulus concentration for L-proline or L-alanine was linear in a relatively wide concentration range, showing a tendency for the response to be saturated at higher concentrations. The results of this study indicate that the amino acids are the most potent gustatory stimuli in the Japanese minnow among various chemicals so far tested including salts, sugars, quinine-HCl and ribonucleotides.

Gustatory responses | Amino acids | High sensitivity | Minnow | Fish
---------------------|------------|-----------------|--------|------

RECENT electrophysiological studies have revealed that amino acids are potent stimuli for the fish gustatory [2, 4, 9, 19, 30] or olfactory receptors [5, 9, 14, 26, 28]. Behavioral studies have shown that some amino acids play an important role in evoking the feeding behavior in certain fishes [3, 7, 10, 14, 17, 27]. Thus, amino acids have been recognized as a class of important chemosensory stimuli.

Data from previous studies in various species of fish indicate that the sensitivity of gustatory receptors to amino acids and their relative stimulatory effectiveness may vary considerably from species to species [2, 4, 9, 25, 30]. Caprio [4,5] has extensively studied the olfactory and gustatory responses to amino acids and related compounds in the channel catfish, Ictalurus punctatus, in order to distinguish between the olfactory and gustatory functions in the same species. He has shown a higher sensitivity for taste than for smell in addition to the difference in the chemospecificity of both systems to amino acids. The lowest gustatory and olfactory thresholds for the most effective stimulus are estimated to be $10^{-1.5} \text{ M}$ for L-alanine and $10^{-0.8} \text{ M}$ for L-cysteine, respectively [5]. A similar high sensitivity of gustatory receptors to amino acids has been reported in the eel, Anguilla japonica, by Yoshi et al. [30]. The lowest threshold of the most potent stimuli such as L-arginine or glycine is estimated to range from $10^{-9}$ to $10^{-8} \text{ M}$. From these results, it is suggested that the gustatory sense may serve as a distance sense for the amino acids in the channel catfish and eel [5,30].

Previously, we revealed that the Japanese minnow palatal receptors exhibit broad responsiveness to various classes of chemicals, corresponding to the high density of taste buds on the upper lip and anterior palate [18,20]. The present study was designed to determine the sensitivity and the response-concentration relationship of gustatory responses to amino acids in the Japanese minnow. Preliminary results have been published elsewhere [18].

METHOD

Material

Eighty-seven Japanese minnows, Pseudorasbora parva, used in this study and weighing 2 to 4 g were caught in a local lake. The fish were maintained in large tanks containing well water in a temperature controlled (20°C) room illuminated from 0800-2000 hr. Commercial fish food was given daily to the fish.

Gustatory Nerve Preparation and Recording System

The fish were immobilized with gallamine triethiodide (Flaxedil, 0.2 mg/10 g body weight, IP) after anesthesia with tricainemethane sulphonate (1:7000 dilution). The methods for obtaining the facial nerve (ramus palatinus) and for recording the neural activity were the same as those described previously [18]. Briefly, after removing the eye ball, the nerve was exposed and freed from surrounding connective tissue. The nerve bundle was transected and its peripheral end was placed on bipolar Ag-AgCl wire electrodes. The electrical activity of the nerve bundle was amplified with an r.c. amplifier and led into an electric sum-
TABLE 1
RELATIVE STIMULATORY EFFECTIVENESS OF VARIOUS AMINO ACIDS AND THEIR DERIVATIVES TESTED AT 10^{-3} M ON THE MINNOW CHEMORECEPTORS SUPPLIED BY THE RAMUS PALATINUS FACIALIS

<table>
<thead>
<tr>
<th>Rank</th>
<th>Chemicals</th>
<th>% Relative effectiveness ± SD</th>
<th>No. of tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L-alanine ethyl ester-HCl</td>
<td>107.4 ± 17.5</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>L-alanine methyl ester-HCl</td>
<td>102.8 ± 22.5</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>L-proline</td>
<td>100 (arbitrary)</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>L-lysine-HCl</td>
<td>83.5 ± 25.5</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>L-alanine</td>
<td>78.5 ± 10.8</td>
<td>28</td>
</tr>
<tr>
<td>6</td>
<td>DL-alanine</td>
<td>73.3 ± 10.2</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>L-arginine-HCl</td>
<td>71.4 ± 16.1</td>
<td>9</td>
</tr>
<tr>
<td>8</td>
<td>D-proline</td>
<td>66.7 ± 10.4</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>DL-alanyl-DL-methionine</td>
<td>64.2 ± 11.2</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>L-cysteine</td>
<td>60.5 ± 11.3</td>
<td>20</td>
</tr>
<tr>
<td>11</td>
<td>L-serine</td>
<td>54.1 ± 10.8</td>
<td>17</td>
</tr>
<tr>
<td>12</td>
<td>DL-alanyl-L-alanine</td>
<td>42.8 ± 10.7</td>
<td>5</td>
</tr>
<tr>
<td>13</td>
<td>L-phenylalanine</td>
<td>41.5 ± 11.8</td>
<td>14</td>
</tr>
<tr>
<td>14</td>
<td>D-alanine</td>
<td>40.5 ± 10.3</td>
<td>5</td>
</tr>
<tr>
<td>15</td>
<td>D-alanine</td>
<td>39.8 ± 9.5</td>
<td>5</td>
</tr>
<tr>
<td>16</td>
<td>Aspartic acid-Na</td>
<td>39.6 ± 13.8</td>
<td>9</td>
</tr>
<tr>
<td>17</td>
<td>DL-alanyl-DL-serine</td>
<td>39.2 ± 9.9</td>
<td>5</td>
</tr>
<tr>
<td>18</td>
<td>L-threonine</td>
<td>38.0 ± 8.4</td>
<td>10</td>
</tr>
<tr>
<td>19</td>
<td>DL-alanyl-glycine</td>
<td>37.8 ± 8.3</td>
<td>5</td>
</tr>
<tr>
<td>20</td>
<td>Glutamic acid-Na</td>
<td>37.7 ± 11.6</td>
<td>10</td>
</tr>
<tr>
<td>21</td>
<td>Glycine</td>
<td>34.7 ± 11.0</td>
<td>15</td>
</tr>
<tr>
<td>22</td>
<td>L-valine</td>
<td>32.6 ± 12.2</td>
<td>9</td>
</tr>
<tr>
<td>23</td>
<td>L-Taurine</td>
<td>27.9 ± 10.4</td>
<td>14</td>
</tr>
<tr>
<td>24</td>
<td>L-methionine</td>
<td>27.3 ± 13.8</td>
<td>15</td>
</tr>
<tr>
<td>25</td>
<td>Betaine</td>
<td>27.0 ± 9.4</td>
<td>5</td>
</tr>
<tr>
<td>26</td>
<td>L-tyrptophan</td>
<td>24.8 ± 5.0</td>
<td>5</td>
</tr>
<tr>
<td>27</td>
<td>L-leucine</td>
<td>24.4 ± 8.7</td>
<td>5</td>
</tr>
<tr>
<td>28</td>
<td>L-histidine</td>
<td>23.3 ± 8.8</td>
<td>8</td>
</tr>
<tr>
<td>29</td>
<td>L-isoleucine</td>
<td>19.8 ± 7.7</td>
<td>8</td>
</tr>
</tbody>
</table>

Stimulants and Their Delivery System

Chemicals tested are listed in Table 1. All were dissolved in distilled water (D.W.). The pH of D.W. used was adjusted to 5.7±0.1 by aeration through a water filter at 20°C, since the gustatory receptors of the minnow were sensitive to CO₂ in solution [29]. Final pH of solutions of neutral chemicals thus was near 6.0. Acidic and basic amino acids were employed in the monosodium salt- and monohydrochloride-form, respectively. The pH of solutions of these amino acids was also near 6.0.

The previous methods of stimulus application [18] were considerably modified in this study. The stimulant delivery apparatus was a combination of silicon tubes, two teflon three-way stopcocks and two glass funnels. Distilled water stored in one funnel was allowed to flow through the flowline at the rate of 5 ml/min into the oral cavity throughout the experiment. Subsequently, most of the water was perfused over the gills. For stimulating gustatory receptors, a stimulus solution stored in the other funnel was introduced to the continuous flow of D.W. by turning a stopcock thereby releasing the test solution for 5 sec. During this period, the flow rate did not change. Dilution of the stimulus was determined by photodensitometry of dye or RNA solutions. Each stimulus was diluted to 40% of its original concentration. The pulse duration was approximately 15 sec as measured at 37% of the peak height. The use of a flow rate of over 5 ml/min did not bring about any significant difference in the magnitude of the summated response. The interstimulus interval was 4 min for lower concentrations (under 10^{-4} M) and was made longer (up to 15 min) for higher concentrations. The standard test solution, 10^{-3} M proline, and the control solution, D.W., were applied regularly to check gustatory responsiveness in the fish.

RESULTS

The palatine nerve, which receives fibers only from the communis root [15,20], was very sensitive to mechanical...
GUSTATORY RESPONSES TO AMINO ACIDS

FIG. 1. Typical summated gustatory responses to various concentrations of L-proline obtained from the palatine nerve of the Japanese minnow. Arrow under each trace indicates the beginning of stimulation.

FIG. 2. Magnitudes of summated responses to various injected concentrations of L-proline (open triangle), L-alanine (open circle) and L-serine (solid circle) plotted against log concentration of the stimulus. Each value represents a mean of 9 (Pro, Ala) and 3 (Ser) experiments and is exhibited relative to the magnitude of the response to 10⁻³ M proline. Abcisal values corrected for dilution. Vertical bars, mean ± SD. C, control response to distilled water.

stimuli in addition to chemical ones as in the case of other trigemino-facial complexes [4, 16, 21]. A fine stream of D.W. over the upper lip or a slight touch with a small glass probe immediately caused a noticeable response capable of masking a response due to chemical stimulation. By using the constant flow system, however, the mechanical sensitivity was easily eliminated. The response appeared about 7 sec after the 5 sec stimulus introduction into the flowline. No appreciable response was found in the stimulation with D.W. (Fig. 1). Phasic mechanical responses were sometimes observed only when the stopcock was turned to initiate the test or D.W. flow. But these mechanical responses were completely separated from the chemical responses.

Figure 1 illustrates the summated response to L-proline of varying concentrations. The summated responses at lower concentrations are characterized by an initial slow rising phase, whereas at higher concentrations the rising phase becomes steeper.

Figure 2 illustrates concentration-response relationships for L-proline (Pro), L-alanine (Ala) and L-serine (Ser). The magnitude of the summated responses to logarithmically increasing concentrations of each stimulant is presented as a percent of that produced by Pro of 10⁻³ M. As seen in Fig. 2, the threshold concentration for Pro was lowest of the three amino acids tested, although it varied to some extent among 9 preparations. In 4 of the preparations the introduction of 10⁻⁸ M Pro consistently elicited detectable responses (Fig. 1) but in the others it failed to cause any response. The response magnitude increased exponentially with logarithmic increase of the stimulus concentration from threshold to 10⁻⁵ M, and at higher concentrations it deviated from the exponential function. A similar concentration-response relationship was also found for Ala or Ser over a wide concentration range. However, Ser did not show any significant deviation from an exponential function up to 10⁻¹ M. After application of the highest concentration of each stimulus to the receptors, their responsiveness was always checked with the standard solution (Pro 10⁻³ M). No significant change in either the standard response magnitude or the baseline activity was observed. The introduction of Ala of below 10⁻⁸ M or of Ser of below 10⁻⁴ M produced no appreciable responses.

The mean response magnitude at each concentration shown in Fig. 2 is replotted in log-log coordinates in Fig. 3. The straight lines in the figure are fit by least square methods within a certain concentration range. A linear relationship between the log response magnitude and the log stimulus concentration was noticed over five log-units for Pro, six log-units for Ala and seven log-units for Ser. Thus, the relationship appears to denote a power law function over a wide concentration range. The exponents of the power function are 0.17 for Pro and 0.12 for Ala and Ser. The intersection of the line with mean control level is referred to as the threshold. Thresholds were calculated to range from 10⁻¹¹ to 10⁻¹⁰ M for Pro, from 5×10⁻¹¹ to 5×10⁻¹⁰ M for Ala, and from 10⁻⁸ to 10⁻⁸ M for Ser, respectively.
FIG. 3. Power function relationship between the magnitude of response to amino acids and the concentration. Each value represents a mean of 3 or 9 experiments, which is shown in Fig. 2. The straight lines were fit by the least squares method: log R = 0.17 log C + 2.85 for L-proline (open triangle), log R = 0.12 log C + 2.33 for L-alanine (open circle), log R = 0.12 log C + 2.13 for L-serine (solid circle). Abscissal values corrected for dilution. C, control response to distilled water.

FIG. 4. Summated responses of the minnow palatine nerve to 10^{-3} M of various substances dissolved in distilled water. Asp, aspartic acid (pH 3.8), Asp-Na, aspartic acid monosodium-salt; Glu, glutamic acid (pH 3.9); Arg, arginine (pH 9.8); Arg-HCl, arginine monohydrochloride; Lys, lysine (pH 9.6); Lys-HCl, lysine monohydrochloride, Pro, proline. The pH of the neutral chemicals was near 6.0.

FIG. 5. Effects of prior application of one stimulus to the minnow palatine receptors on the response to another stimulus. A second stimulus was applied 10 sec after beginning of the first stimulation. It is noted that prior application of proline on the receptors resulted in a complete cross adaptation of the receptor to alanine while the other pairs of stimuli caused a distinct second peak after a first response. The chemicals were tested at 10^{-3} M.

Summated responses to 22 amino acids, 6 related compounds and betaine at the same concentration (10^{-3} M) were examined and the results are summarized in Table 1. The minnow palatal receptors responded to all of the chemicals tested. The relative stimulatory effectiveness of acidic and basic amino acids was as follows: Asp = 125.2±28.0, n=15; Glu = 101.9±13.2, n=15; Arg = 29.5±9.3, n=17; Lys = 27.1±8.5, n=16. However, the solutions of these amino acids at 10^{-3} M were highly acidic or alkaline (Asp pH 3.8, Glu pH 3.9, Arg pH 9.8, Lys pH 9.7), and thus pH contributed to the responses. In order to estimate specific molecular effects of the 4 chemicals on the receptors, the monosodium salt-form of acidic amino acids and the monohydrochloride-form of basic amino acids were also tested. The pH of these solutions was near 6.0 as that of the others listed in Table 1. Figure 4 shows typical records of the summated responses. The two acidic amino acids were more stimulative than their monosodium-forms (Asp-Na, Glu-Na). On the other hand, the two basic amino acids were less effective than their monohydrochloride-form (Arg-HCl, Lys-HCl). This result suggests that the basic amino acids may be more effective at pH near 6.0 than at pH near 9.8, assuming that chloride ions in the Arg-HCl or Lys-HCl solution do not have a strong stimulating effect.

In an attempt to estimate the contribution of the chloride ions to the response to Arg-HCl or Lys-HCl, the extent of cross adaptation between chlorides (HCl, NaCl) and the above two stimulii was examined in 10 preparations. The experimental procedures were as follows. The first stimulus of a pair was introduced to the flowline for 10 sec and was followed by the second stimulus stored in a separate funnel. After the 10 sec introduction of the second stimulus the lip region was again continuously rinsed with D.W. for 10 min or greater and then another pair was applied. Figure 5 shows a typical record of the cross adaptation experiments. As seen
in the lower trace, application of 10^{-3} M Lys-HCl following
stimulation with 10^{-3} M HCl or NaCl produced a distinct
second peak. However, its magnitude measured from a level
of the integrated pattern immediately before the appearance
of the second response was affected considerably by the
previous presentation of the chlorides, indicating that cross
adaptation occurred to a good extent between these pairs.
The magnitude of the second response was reduced by ap-
proximately 35% of the response magnitude of Lys-HCl
when it was applied alone. Similar results were also obtained
between the chlorides and Arg-HCl. From these results pre-
sented above, it can be said that the stimulating effectiveness
of Lys-HCl or Arg-HCl (10^{-3} M) is ascribed to a certain
extent to the dissociated molecules of basic amino acids.
However, it is difficult to determine the precise extent.
Smith and Frank [24] pointed out that the amount of cross
adaptation between two stimuli indicate the extent to which
they compete for the same receptor site. They also pointed
out that prolonged stimulation may render taste receptor
cells less excitable if taste receptors were relatively non-
specific and, therefore, that adaptation to one stimulus
would prevent the receptor from responding to others which
fill sites on the same cell. If cross adaptation which occurred
between the chlorides and the hydrochloride-form of basic
amino acids in the minnow (Fig. 5) was caused to some ex-
tent by the latter case, true stimulating effectiveness of basic
amino acid molecules in their hydrochloride-form solutions
could be greater than that expected from the cross adapta-
tion experiments.

As seen in the upper traces of Fig. 5, aspartic acid (10^{-8}
M, pH 3.8) after HCl (10^{-3} M, pH 3.0) produced also a dis-
tinct second peak, indicating that the stimulating effective-
ness of the acidic amino acids can be ascribed not only to low
pH of the solutions but also to the molecules of these amino
acids. On the other hand, alanine after proline elicited no
detectable second peak. When the relative stimulatory effec-
tiveness of amino acids was compared with each other at
a pH near 6.0, the most effective of 18 L-amino acids were
proline > lysine-HCl > alanine > arginine-HCl > cysteine >
serine (Table 1). A clear difference was found in the magnitude of the re-
sponse between enantiomeric pairs of alanine or proline; the
L-isomer was more effective than its D-form. The response
to racemic mixtures (DL-alanine) was almost identical with
that to the corresponding L-isomer. The L-amino acids (L-
Ala) was far more effective than the β amino acid (β-Ala).
The estification of the carboxyl group (L-alanine ethyl ester,
L-alanine methyl ester) resulted in a greater response magni-
itude than that to the corresponding free amino acids (L-
Ala). Some dipeptides consisting of DL-alanine and another
amino acid were also stimulatory, but were not as effective
as the most stimulatory amino acid residue in the molecule.

**DISCUSSION**

The acuity of the gustatory sense has been well demon-
strated in the European minnow, *Phoxinus phoxinus* by
Glasser who examined behavioral responses to various taste
substances [8]. The behavioral taste thresholds for NaCl,
quinine-HCl, sucrose, and saccharin-Na are approximately
4×10^{-5} M, 4.1×10^{-8} M, 1.2×10^{-5} M and 6.5×10^{-7} M, re-
spectively. Except for NaCl, these behavioral thresholds
found in the European minnow are greater than the elec-
rophysiological ones for the corresponding substances in
the Japanese minnow [18]. The present study indicates that
the Japanese minnow palatal receptors are extremely sensi-
tive, especially to Pro and Ala. The threshold for the former
was estimated to be between 10^{-11} and 10^{-10} M, which
is lower than any other threshold determined so far in the
above two species of minnow. Thus, among various classes of
substances tested including salts, sugars, ribonucleotides
and others [18], the amino acids are the most potent stimuli
for Japanese minnow.

Fish have three peripheral gustatory systems: facial, glos-
sopharyngeal and vagus systems. Among them, the facial
system related to taste buds located on the external skin and
the mucous membrane covering the anterior mouth cavity
may function to localize food and initiate the start of
feeding activity [1]. The acuity of minnow palatal receptors
presented here suggests that the facial gustatory system of
this fish having taste buds with a high density on the lip
region [20] may serve as a distance sense for the amino acids.
A similar high sensitivity of gustatory receptors to amino
acids has been shown in the barbels of channel catfish [4,5]
and the anterior palate of eel [30]. In both cases, thresholds
for the most potent amino acids were estimated to be less
than 10^{-4} M. However, the results of these two and the pres-
tent studies differ in the relative order of stimulatory effec-
tiveness of the amino acids tested. The five most effective of
the L-amino acids tested at 10^{-4} M were Ala > Arg > Ser >
Abu (α-amino-butyric acid) > Gln (glutamine) in the channel
catfish [4] and Arg > Gly > Ala > Pro > Lys in the eel [30].
In the Japanese minnow the most highly stimulatory amino
acids tested at 10^{-8} M were Pro > Lys-HCl > Ala > Arg-HCl
> CysH (L-cysteine). Thus, the relative stimulatory effec-
tiveness of amino acids on the gustatory receptors varies
from species to species, possibly reflecting different feeding
habits of the fish.

Another contrast among the three species possessing high
taste acuity to amino acids is the relationship between the
magnitude of the summated responses recorded in a whole
nerve preparation and the concentration of the amino acid
stimuli. In the channel catfish [4,5], the relationship is a
power function, which is similar to those found for the olfac-
tory neural responses of some fishes [5,28]. For example, the
response magnitude to Ala increased exponentially with
logarithmic increase in the stimulus concentration from
threshold (averaged 10^{-11.5}±1.7 M) to 10^{-2} M. Caprio and
Tucker [6] further analysed this relationship in 26 single fiber
preparations of the catfish. They observed that the fre-
quency of impulse discharges during stimulation in most of
the fibers is a power function although the concentration
range which each fiber can respond to is more variable than
found for the whole nerve preparation. In the eel, on the
other hand, the relationship is a logarithmic function [30].
For example, the response magnitude to Arg or Gly in-
creased approximately linearly with logarithmic increase in
the stimulus concentration from 10^{-8} to 10^{-2} M. In the above
two species no tendency of saturation of the response was
observed at higher concentrations.

The present results show that the relationship in the min-
now can be expressed by a power function rather than a
logarithmic function for a relatively wide concentration
range (Figs. 2, 3), which is in part similar to that found in the
channel catfish [5]. However, it is characteristic of the min-
now that the response magnitudes to Pro and Ala tend to
be saturated above 10^{-8} M and 10^{-4} M, respectively. It is not
likely that this tendency of saturation is simply attributed
injurious effects of high concentrations of the stimuli on the
receptors, since the response magnitude to Ser is well fit by a
power function even at $10^{-1}$ M. Further, after stimulation with such a high concentration, responsiveness of the receptors was well maintained. Considering the amino acids concentration found in extracts of various organisms such as clams or shrimp \cite{22,23}, $10^{-1}$ to $10^{-4}$ M of proline, appears to be regarded as within the natural range of concentration which the fish can taste in their environment. A similar saturation of the amino acid responses at approximately $10^{-4}$ M was observed in the olfactory bulbary recordings from the rainbow trout, *Salmo gairdneri* \cite{12}.

The pH is itself a factor of stimulating effectiveness for the taste receptors \cite{2,16,18,25}. Also, the pH should be considered to have effects on the stimulating effectiveness of amino acids since they are amphotiles. This was demonstrated earlier in the olfactory bulbary responses of rainbow trout to amino acids \cite{13}. Contrastingly, it has been reported that no significant difference was observed in the taste response of eel palatine receptors to $10^{-5}$ M glycine at pH 5.0 and 9.0, \cite{30}. The present study (Figs. 4, 5) indicates that Arg-HCl and Lys-HCl (pH near 6.0) are more stimulatory for the palatal receptors of minnow at $10^{-3}$ M than Arg and Lys (pH near 9.7). Further, a distinct second peak is produced by stimulation with Lys-HCl or Arg-HCl ($10^{-3}$ M) followed by HCl or NaCl ($10^{-2}$ M). This result suggests that the responses to the basic amino acids may be enhanced by lowering the pH of the stimulus solution. Arg at a pH near or below 8.0 is shown to be one of the highly stimulative amino acids for gustatory receptors of channel catfish \cite{4}, eel \cite{30}, red sea bream and mullet \cite{9}. Except in catfish, a similar tendency is also seen for Lys in these fishes.

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