Gustatory Response in the Young Yellowtail *Seriola quinqueradiata*^{*1}

Iwao HIDAKA,^{*2} Tadashi OHSUGI,^{*2} and Yoshifumi YAMAMOTO^{*2}

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The sensitivity of the palatal receptors of young yellowtails *Seriola quinqueradiata* to extracts of animals were studied by recording the electrical neural responses from the anterior palatine nerve.

The palatal receptors responded to amino acids, betaine, and nucleotides and related substances. Of the 20 amino acids tested, proline had the lowest threshold of around 10^{-6} M. The threshold for tryptophan, the next most effective amino acid, was around 10^{-4} M, while many amino acids were ineffective at 10^{-2} M and thus, a relatively narrow amino acid response spectrum was seen at 10^{-2} M. Among the effective nucleotides and related substances were adenosine, inosine, AMP, ADP, ATP, IMP and UMP. Sodium salts of fumaric acid, malic acid and succinic acid, and urea and other nitrogenous excretory substances were ineffective at 10^{-2} M. Sucrose at 0.5 M was also ineffective.

As the yellowtail *Seriola quinqueradiata* is an active predatory fish, it might be expected that vision would be the most important sense involved in its feeding behavior. However, recent experiments suggest that chemical stimuli is also of importance in the feeding behavior of this species. Takeda and his colleagues found stimulation of the feeding response by extracted components of food animals of young yellowtails.¹⁾ While casein-based artificial food alone is not accepted by the fish, it is accepted when enriched with amino acids, nucleotides etc. HARADA²⁾ has also observed that juvenile yellowtails are attracted to the extracts of various organisms in solutions. These observations indicate the role of a chemosensory response in the feeding behavior of young yellowtails at least in captivity, although whether the chemical sense is smell or taste is not known from the above studies.

This work was undertaken to elucidate the sensitivity of the palatal taste receptors of the yellowtail to a variety of chemicals of biological significance.

Materials and Methods

The present experiments were performed in August, 1978 and 1979, at Usa Marine Biological

Institute, Kochi University, Usa, Kochi Prefecture. The experiments in Figs. 2 and 3A were conducted in June, 1980, at the Fisheries Research Laboratory at Zaga Island, Mie University, Wagu, Mie Prefecture.

A total of 49 yellowtails *Seriola quinqueradiata* weighing 69 to 160 g were used. They had been originally caught offshore by fishermen, and fed for one to two months on the sand eel *Ammodytes personatus*, horse mackerel *Trachurus japonicus*, and/or chub mackerel *Scomber japonicus* before experimentation. The fish were lightly anesthetized with MS222 (0.2 g/l) and then immobilized by intramuscular injection of gallamine triethiodide (10 mg/kg of body weight). The fish was then inclined on its back with its head slightly higher than its tail. Throughout the experiment sea water was perfused through the mouth over the gills. After removing the eye, the ramus palatinus facialis running toward the anterior region of the palate was isolated and cut off from the central connection. The electrical neural activity was recorded as usual by using an electronic integrator (Nihonkohden, RJG 4022SS, time constant 0.02 s).

Test stimulants were dissolved in artificial sea water. The solutions of arginine, lysine, adenosine-5'-diphosphate and adenosine-5'-triphosphate

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^{*2} Faculty of Fisheries, Mie University, Tsu, Mie 514, Japan (日高馨夫・大杉 正・山本良文: 三重大学水産学部).

were adjusted to pH 8 with hydrochloric acid and sodium hydrochloride. The stimulus solutions were applied in constant flow to the innervated area of the palate with the aid of a glass tube inserted in the mouth. Artificial sea water was continuously flowed between stimulations. The nucleotides used were all in the form of disodium salt and together with the nucleosides and D-ribose, were commercial preparations by Kohjin Co., Ltd. The other chemicals were by Nakarai Chemicals, Ltd. (reagent grade). The experiments were performed at room temperature ranging from 20–22°C.

Results

The palatal receptors of the yellowtail responded and were highly sensitive to substances including amino acids, betaine, nucleosides and nucleotides. They also showed a high sensitivity to dilution of sea water, on which a separate report has been published.³⁾

Fig. 1 shows an example of the recorded neural responses (summed) to stimulation of the palate by various chemicals. The summed neural response usually peaked shortly after application of the stimulus solutions and attenuated without yielding any appreciable plateau even to stimulations by strong solutions of 10^{-2} M. In the following, responses to different stimuli are compared with each other by measuring their maximum amplitude.

Amino Acids

Fig. 2 shows the magnitudes of the summated responses to 19 amino acids at concentrations of 10^{-2} M and 3×10^{-2} M. As seen in the figure, only a few of the amino acids were appreciably effective

Fig. 1. Summated responses of the palatine nerve on application of (a) proline, (b) betaine, (c) tryptophan, (d) valine, (e) methionine, (f) glycine and (g) AMP at 10^{-2} M to the palate. The bar under each response indicates the application period of stimulus.

Fig. 2. Bar graphs showing mean values of the summated responses to amino acids and betaine relative to that of 10^{-2} M tryptophan, A, for 10^{-2} M solutions; B, for 3×10^{-2} M solutions. The horizontal lines represent standard deviation. The figures in the parentheses indicate the number of fish tested.

at 10^{-2} M. Examination of the dose-response relationship at a lower concentration range showed that the thresholds for these amino acids, except proline, are not so low. The threshold to proline appeared to be around 10^{-6} M, while that of tryptophan, the 2nd most effective amino acid, appeared to be around 10^{-4} M and that of other amino acids were still higher (Fig. 3). Some of the amino acids which were ineffective at 10^{-2} M could elicit a more or less positive response at 3×10^{-2} M (Fig. 2). However, some were still ineffective at this concentration. Sodium aspartate and glutamate elicited no appreciable response even at 0.1 M. Thus, the sensitivity of the yellowtail to amino acids seems to be generally lower than that of other species previously investigated such as the channel catfish *Ictalurus punctatus*,⁴⁾ eel *Anguilla japonica*,⁵⁾ mottosugo (minnow) *Pseudorasbora parva*⁶⁾ or carp *Cyprinus carpio*⁷⁾; in these species, many amino acids are effective at 10^{-6} M and even lower concentrations.

The relative stimulatory effectiveness of amino acids for the yellowtail taste receptors (Fig. 2) also

Fig. 3. Dose-response curves for (A) amino acids and betaine, and (B) nucleotides. Mean values of the magnitudes of response relative to 10^{-2} M tryptophan are shown. The figures in the parentheses indicate the number of fish tested. The vertical bars on the plots in (A) represent standard deviation.

differs from that of other species; while proline is one of the most effective amino acids in many other species,⁴⁻¹⁰ the high rank of tryptophan in the yellowtail is not general. Tryptophan ranks relatively high in the channel catfish, but not in other species such as the eel, mottosugo, and red seabream *Chrysophrys major*.¹¹ The low sensitivity to glycine (Figs. 1 and 2) is characteristic of the yellowtail receptors, whereas taste receptors of several other species are highly sensitive to it.^{4,5,9,11} Thus the present results demonstrate unique features of the amino acid response spectrum of the yellowtail.

D-isomers of amino acids (D-alanine, D- α -amino butyric acid, D-leucine and D-valine) and β -alanine were also tested with two preparations, but none of them were effective at 10^{-2} M.

Betaine

Betaine (trimethylglycine) is widely found in tis-

suces of potential food animals of fish and is found in large quantities in invertebrates such as mollusks and crustaceans.¹⁰ It has been reported as having a stimulatory effect on feeding in some fish, whether applied alone or mixed with amino acids,^{12,14-20} and is also a potent stimulant for the taste receptors of several fish species.^{5,9,10,11,21} In the present study also, betaine was an effective stimulant with the threshold of around 10^{-6} to 10^{-5} M (Fig. 3).

Nucleosides and Nucleotides

The yellowtail taste receptors responded well to adenosine, inosine, adenosine-5'-monophosphate (AMP), adenosine-5'-diphosphate (ADP), adenosine-5'-triphosphate (ATP), inosine-5'-monophosphate (IMP) and uridine-5'-monophosphate (UMP). No appreciable responses were elicited by uridine and citidine-5'-monophosphate (CMP) at 10^{-2} M in five preparations tested. Fig. 4 shows the dose-response curves for AMP, ADP, ATP, IMP and UMP obtained with two to three preparations. AMP, ADP, IMP and UMP were effective at 10^{-4} M. AMP and ADP could elicit a positive response at 10^{-5} M in two and one preparations, respectively.

The sensitivity of taste receptors to nucleotides and related substances has also been investigated in several other fish species. All nucleotides effective in the present study are also effective in the mottosugo and puffer *Fugu pardalis*.²² In the mottosugo, CMP also is one of the most effective nucleotides. In the seabream and mullet *Mugil cephalus*,¹² none of the nucleosides nor nucleotides tested were stimulatory at 10^{-2} M.

Others

Sodium salts of fumaric acid, malic acid and succinic acid, trimethylamine oxide, creatine, creatinine, urea and ammonium chloride were also tested with one to three preparations each. None of them appeared to be effective at 10^{-2} M. Sucrose at 0.5 M and D-ribose at 10^{-2} M were not effective.

Discussion

The present results demonstrate that the palatal taste receptors of the yellowtail are responsive to various substances. The low sensitivity to many amino acids suggests that the receptors for these amino acids in this fish do not mediate low-level stimulus detection (no response at a distance from the food organisms). The thresholds for some amino acids, however, are well within the

natural physiological range: the anchovy *Engraulis japonicus*, horse mackerel and chub mackerel are potential preys of the yellowtail²³⁻²⁵⁾ and their muscle tissue extracts^{26,27)} contain alanine, for example, at concentrations of 3.4×10^{-8} , 2.4×10^{-8} and 2.9×10^{-8} mole/kg of muscle, respectively, whereas the experimental threshold for alanine was around 10^{-8} M (Fig. 3). Thus, it is possible that the fish taste receptor is excited by some of the amino acids in the prey tissue when biting or after enclosure in the mouth.

The relatively high sensitivity to proline and betaine may be advantageous to the yellowtail for detecting its invertebrate prey. It is known that the yellowtail eats squids and mysid crustaceans²³⁻²⁵⁾ and is easily attracted to the krill *Euphausia pacifica* when in captivity. Proline and betaine are found in both krill and squid in large concentrations of 2×10^{-2} to 8×10^{-2} mole/kg of tissue.²⁸⁻³¹⁾

In their experiments with casein-based food, Takeda and his colleagues, observing young yellowtails, found that the main stimulatory components of krill extract are proline, alanine and methionine. They also report that IMP is mainly responsible for the stimulatory effect of extract of horse mackerel muscle.¹⁾ The large feeding stimulatory effect of IMP and inosine has also been reported for the turbot *Scophthalmus maximus*.³²⁾

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