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Single Fiber Responses in the Palatine Taste Nerve of the Yellowtail *Seriola quinqueradiata*

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Sensitivities to alanine, arginine, proline, tryptophan, valine, betaine, uridine 5′-monophosphate and diluted sea water were studied for 83 single fibers of the palatine taste nerve (ramus palatinus facialis) in the young yellowtail *Seriola quinqueradiata*.

Results of the analysis of the impulse numbers elicited in the first one or first two seconds after the onset of stimulation showed that the sensitivities to the 8 stimulants were not randomly distributed among these fibers: most of the fibers were specifically sensitive to a limited number of stimulants and some were only sensitive to one. Thus, the fibers obtained were classified into five major groups in their sensitivity patterns: (1) fibers responding specifically to tryptophan, uridine 5′-monophosphate and diluted sea water; (2) those responding well to betaine, proline and valine; (3) those responding only to betaine; (4) those responding well to alanine and arginine; and (5) those responding only to alanine.

Recording of the electrical responses from taste nerves in many species of fish show that fish taste receptors have a high sensitivity to tissue components of prey organisms, such as amino acids and nucleotides.[1-15] These investigations also reveal that the response spectra or the sensitivities to various chemicals differ considerably from species to species of fish. This might suggest evolutional development of species' specific taste sense to detect constituents in the tissues of various prey organisms associated with their feeding habits.

Early single fiber studies[16-18] show that fish taste fibers or taste units, like those of higher vertebrates,[19-21] have a multisensitivity, sensitive to more than one quality of chemicals, in rather a random fashion. However, specialist fibers, specifically responding to certain chemicals, have also been reported; for instance, in the facial nerve innervating the snout region of the sea catfish *Plotosus anguillaris* (=*lineatus*) many fibers were found which responded well to the extract of a marine worm but not to other stimulants used.[18] The maxillary branch of the facial nerve of the puffer *Fugu pardalis* contains three disticnct groups of fibers responding specifically to amino acids, nucleotides or hydrochloric acid.[5,6] Fibers highly specific for arginine are also reported for the barbel nerve of the channel catfish *Ictalurus punctatus*.[22] These findngs are evidence of independent receptors for the effective stimulants. Thus, the occurrence of such specific fibers together with the above-mentioned species specific multifiber response spectra seem to suggest a high degree of differentiation of fish taste receptors and diversification of the signal relaying patterns of taste units among fish species.

The yellowtail *Seriola quinqueradiata* has also been shown to have a sensitivity to amino acids and other tissue components of organisms by recording multifiber responses from the palatine branch of the facial nerve.[13] Its response spectrum also is its own and differs from that of the puffer,[23] tigerfish,[14] or other coastal species of teleosts.[8,15] In this study, the sensitivity profiles of single fibers of the same nerve of the yellowtail were analyzed using 8 stimulants. Most of the fibers obtained responded to one or some of them. The sensitivities to them were not randomly distributed among the fibers but highly associated for groups of stimulants. Thus, the fibers obtained were classified into certain groups in their sensitivity patterns.

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Materials and Methods

The experiments were performed at the Fisheries Research Laboratory, Mie University, Zaga Island, Wagu, Mie Prefecture from September to November, 1988. Some supplementary experiments were also performed during the same season of 1989.

A total of 38 yellowtails *Seriola quinqueradiata* weighing 295 g to 640 g were used. They had been originally caught off-shore by fishermen, and fed for three to 5 months on the sand lance *Ammodytes personatus*, chub mackerel *Scomber japonicus* and and an artificial feed by Marubeni Shiryo Co. before experimentation. They were immobilized with muscular or intraperitoneal injection of a muscle relaxtant (pancuronium chloride, 1.0–1.5 mg/kg body weight) and placed inclined on a grooved wooden block on its back. Throughout the experuments, the gills were irrigated with sea water. After removal of the eyeball, the palatine branch of the facial nerve was isolated and cut from the central connection. The electrical activity of the whole nerve bundle was recorded as the summated response[13] using an electronic integrator (Nihon Kohden, RJG 40225S). To obtain small strands with one or two functionally intact fibers, the peripheral nerve end was dissected with forceps and a needle on a small glass mirror inserted in the orbit. A pair of platinum electrodes were used for recording the impulses of the single fibers. The impulse discharges were displayed on an oscillograph (Nihon Kohden, VC-9) and recorded on paper film using a camera (Nihon

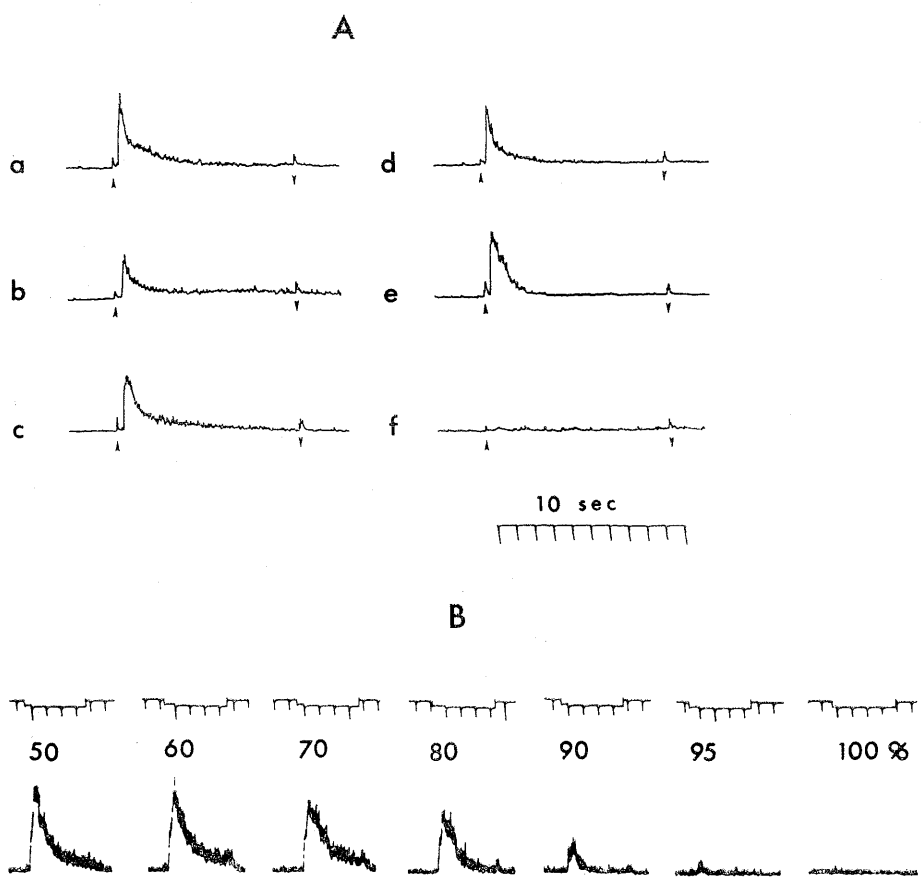

Fig. 1. A: Summated neural responses to amino acids and other chemicals at 10^{-2} M (A) and those to sea water at different concentrations (B) from the palatine branch of the yellowtail *Seriola quinqueradiata*. A: A pair of arrows under each recording indicate the duration of stimulus application. a, proline; b, alanine; c, tryptophan; d, betaine; e, uridine 5′-monophosphate; f, glycine. B: The upper trace in each recording, time maker, in sec and the sustained downward deflection of the base line indicates the duration of stimulus application. A and B were obtained with different preparations.

Kohden, RLG-6101). The stimulus duration was usually three sec and the intervals between stimulations were about one min or more. The palate was continuously rinsed with artificial sea water between stimulations. Stimulants were dissolved in artificial sea water. Of the chemicals used, uridine 5′-monophosphate was from Khojin Co. and the others were from Nacalai Tesque Co. (GR grade). During the periods of the experiments, the temperature of the respiratory sea water ranged 13.8–25.6°C. The room temperature during the experiments was adjusted to the sea water temperature, ranging from 14–24°C. The temperature change during one experiment was kept at ±1°C.

Results

The Nature of the Responses of the Yellowtail Taste Fibers

Seven single chemicals, L-alanine, L-arginine, L-proline, L-tryptophan, L-valine, betaine, and uridine 5′-monophosphate, and 50% sea water were used as test stimulants. All of them have previously been shown to be effective stimulants for this fish.[13,24] The summated response recorded from the palatine nerve on application of any of these stimulants to the palate was phasic in nature, even for high concentrations, and usually attained the maximum magnitude within about one sec and then rapidly declined to some 20 or 30% level or below at two sec after the onset of stimulation[13] (Fig. 1).

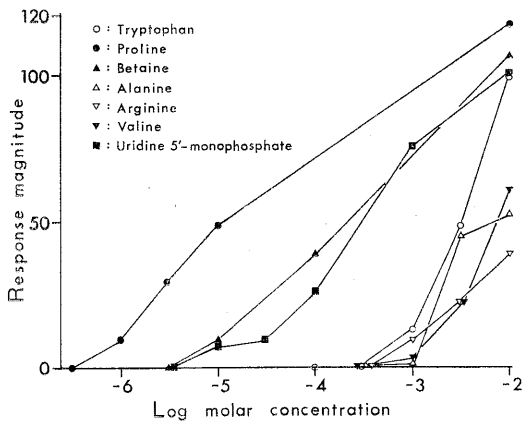

Fig. 2. Dose-response curves for 7 chemicals obtained with a whole nerve preparation. Ordinate, response magnitude in arbitrary units. Abbreviation: Ala, alanine; Arg, arginine; Bet, betaine; Pro, proline; Trp, tryptophan; UMP, uridine 5′-monophosphate; Val, valine.

Therefore, in the following single fiber experiments, the impulse frequencies for the first two sec were mainly analyzed. Spontaneous discharges were quite rare during the recording with single fiber preparations though considereable activities were recognized in the whole nerve preparations. The impulses elicited in two sec immediately before and after the application of artificial sea water as control were respectively 0.0±0.0 (SD) and 0.5±0.7 (SD) on the average of 17 fibers. Accordingly, in the following analyses the responses to stimulants were tentatively treated as "positive" when there were observed two or more impulses during the first two sec after the onset of stimulation. The threshold of the 7 single chemicals tested were 10^{-6} M to 10^{-3} M depending on chemicals.[13] In the present single fiber study, all these chemicals were used at 10^{-2} M, some one order higher concentration for the least effective of the 7, alanine, arginine and valine, than their threshold value in the whole nerve (Fig. 2). The yellowtail also shows a high sensitivity to dilution of sea water[24]: a 3 to 5% diluted sea water may elicit a positive response and the response rapidly increases with further dilution (Fig. 1B). Thus, 50% sea water was nearly as effective as 10^{-2} M tryptohan, one of the most effective amino acids for this fish.

The Sensitivity Patterns of Taste Units

A total of 83 functionally single fibers were isolated. These fibers were confirmed to be "functional" by stimulating the palate with the 8 stimulants singly or in mixtures. Therefore, all the sampled fibers were those at least sensitive to one of the 8 stimulants. Fig. 3 shows an example of the recordings from a preparation which contained two functional fibers, one responding well to uridine 5'-monophosphate, tryptophan and 50% sea water, and the other responding to alanine. Most of the fibers obtained responded specifically to one or some of the 8 stimulants: some fibers responded well to one or some stimulants, while others did so to others. Thus, they fell into certain groups in their sensitivity patterns. Fig. 4 summarizes the sensitivity distributions among the 83 fibers. The fibers were arranged in abscissa in order of impulse numbers elicited during the first two sec after stimulation for, from left, uridine 5′-monophosphate, betaine and then alanine. Fibers responsive to two or more of the three stimulamnts were ordered for the one which gave the largest response. As may be seen in Fig. 4, the fibers obtained were roughly classified into three major groups: uridine 5′-monophosphate

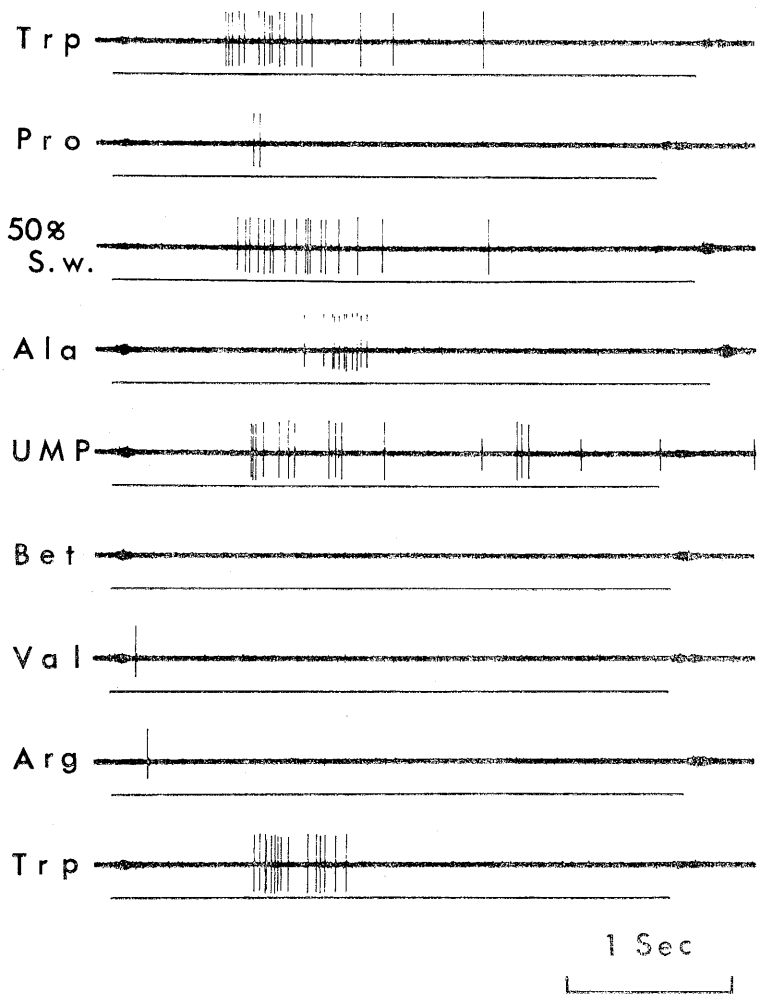

Fig. 3. Recordings of the responses to 8 stimulants in a few fiber preparation, which contained two fibers, one responding well to uridine 5′-monophosphate, tryptophan and 50% sea water, and the other responding to alanine. Similar sizes of spikes are seen for these four stimulants, but the ratio of the upward and downward components of the spikes for the former three differ from that of those for the latter, indicating that the two spikes were from different fibers. Bar under each recording indicates the duration of openning the solenoid valve of the stimulatory apparatus for stimulus solution flow. It took about 0.6 sec for a test solution to reach the palate after opening the valve. Recordings for the 8 stimulants were performed in order. 50% S. w., 50% sea water, and other abbreviations, as in Fig. 2.

sensitive, betaine sensitive, and alanine sensitive groups. Of the 76 fibers in which all the 8 stimulants were tested, 80% were only sensitive to one of the three stimulants: Twenty seven fibers were sensitive to uridine 5′-monophosphate. Of them, one (fiber 38) also responded to betaine and alanine but weakly. Two others (fibers 66 and 70) responded markedly to alanine. In these fibers, however, the responsiveness to uridine 5′-monophosphate itself was very weak. There were 38 betaine sensitive fibers among the 76, of which 24 were sensitive to neither uridine 5′-monophosphate nor alanine. Thirteen fibers responded to alanine, but mostly weakly. Alanine sensitive fibers were 27, of which 12 were sensitive to neither uridine 5′-monophosphate nor betaine. The fibers of the three groups also behaved in the same groups or in subgroups to other stimulants. Thus, they finally fell into 5 major groups in their sensitivity patterns for all the 8 stimulants: Most of the

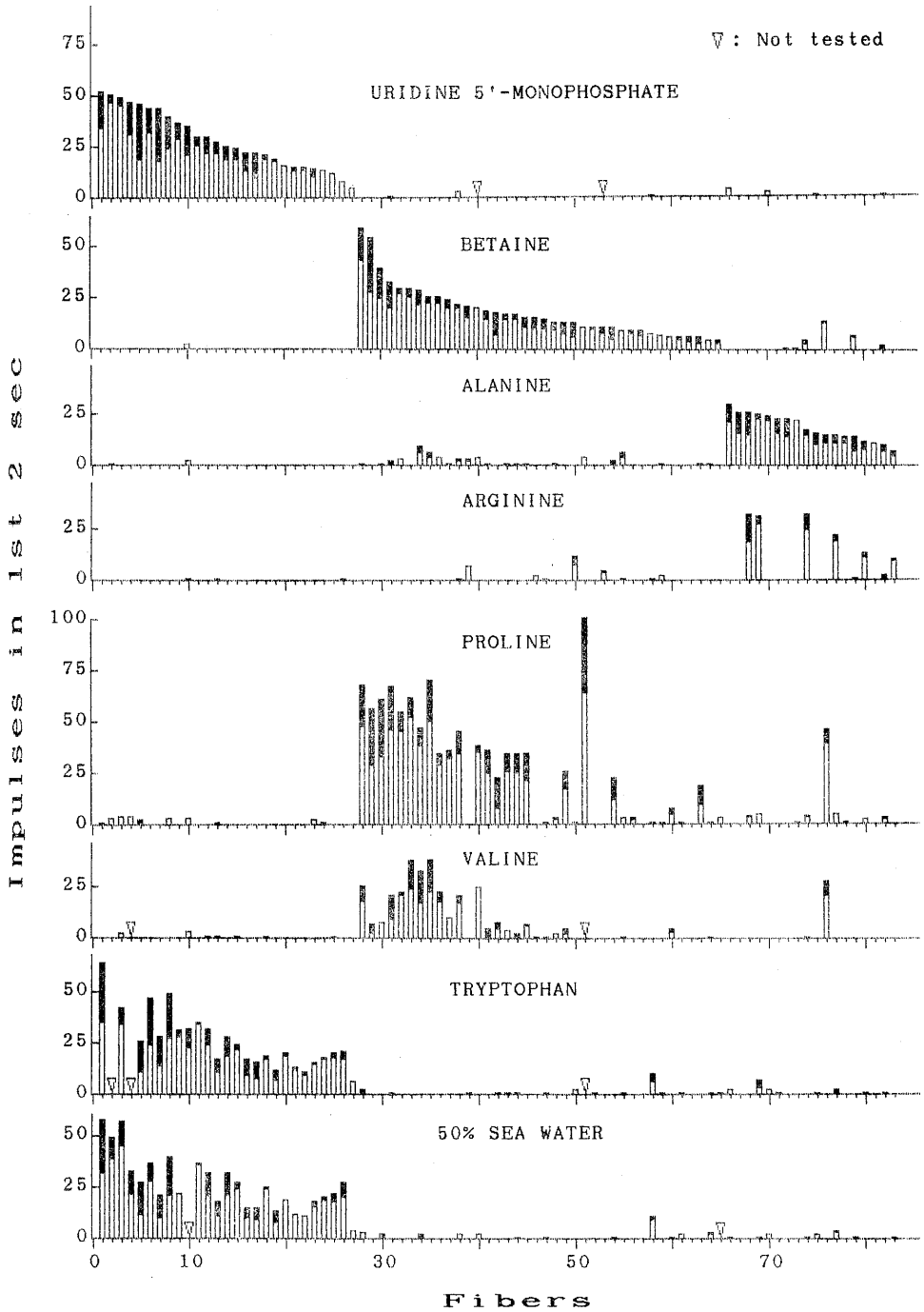

Fig. 4. Bar graph showing the sensitivity distribution for 8 stimulants among 83 palatine fibers. Ordinate, impulse numbers elicited in the first two sec after the onset of stimulation. Open and filled portions of each bar represent impuluses in the first and second sec, respectively. Abscissa, fibers arranged in order of response size for, from left, uridine 5′-monophosphate, betaine and alanine. Fibers responsive to two or more of the three stimulants were ordered for the one which gave the largest response.

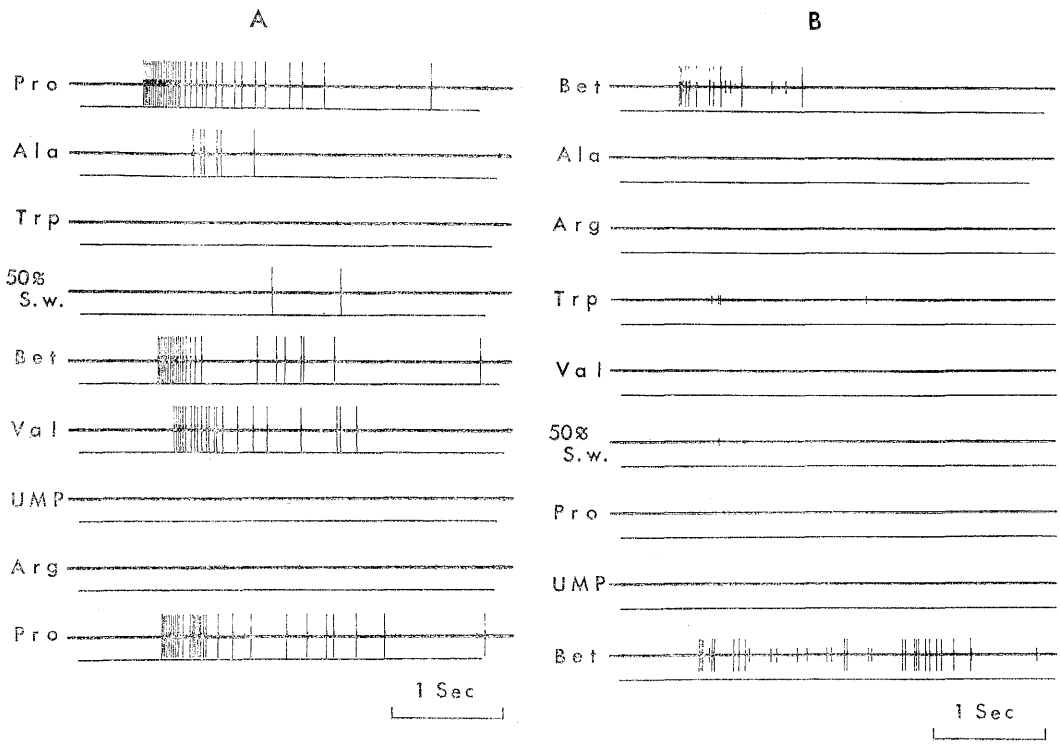

Fig. 5. Recordings from two types of betaine sensitive fibers, one responding well to betaine, proline and valine(A) and the other responding only to betaine (B). 50% S. w., 50% sea water, and other abbreviations, as in Fig. 2. For explanations of the bar under each recording, see Fig. 3.

uridine 5′-monophosphate sensitive fibers were also sensitive to tryptophan and 50% sea water (type 1). The majority of the betaine sensitive fibers also responded well to proline and valine (type 2) (Fig. 5A) while some were betaine specific and responded to neither of them (type 3) (Fig. 5B). In the former type fibers, there was a tendency that fibers responding well to betaine also responded well to proline and a significant correlation was observed in the impulse frequencies between the two stimulants. The responses of the type 3 fibers to betaine were in general weaker than those of the type 2 fibers: the impulse numbers elicited in the first one sec was 24 ± 14(SD) and 10 ± 5(SD), for the type 2 and type 3 fibers, respectively. This suggested that the sensitivity of the latter fibers to proline might also be lower than the former fibers. However, their failure to respond to it strongly suggested their insensitiveness to it because its concentration used was 4 orders larger than its threshold obtained with the whole nerve preparations[13] (Fig. 2) and moreover the average impulse frequency for it in the type 2 fibers was even higher than that for betaine.

Alanine sensitive fibers were divided into two subgroups: fibers sensitive to arginine (type 4) and those highly specific for alanine (type 5) (Fig. 2). There were also observed some fibers responding to arginine and betaine. In type 1 group, fibers responding well to uridine 5′-monophosphate also tended to repsond well to both tryptophan and 50% sea water and thus exhibited similar across-fiber response patterns for the three stimulants (Fig. 4). Similar correlations were also observed between the responses to proline and valine and and those to alanine and arginine in the type 2 and type 4 fibers, respectively.

Discussion

The single fibers in the palatine branch of the facial nerve of the yellowtail obtained were highly specific for stimulants and fell into 5 major groups in their sensitivity patterns for the 8 stimulants. Fibers of the type 1 group were highly specific for uridine 5′-monophosphate, tryptophan and 50% sea water. Almost no fibers in the other groups did respond markedly to these three stimulants.

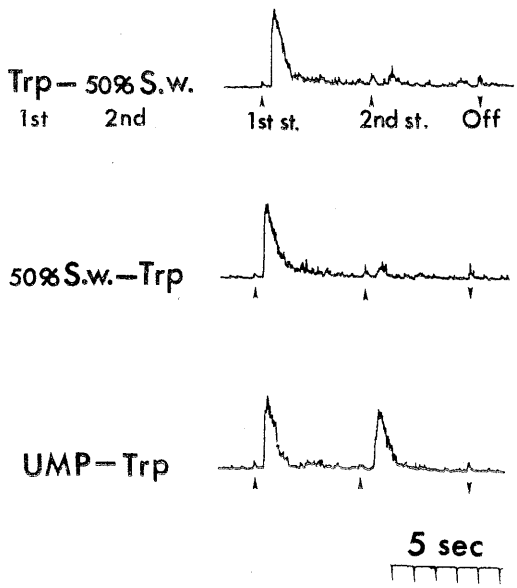

Fig. 6. Cross-adaptation experiments for combinations of uridine 5′-monophosphate, tryptophan and 50% sea water. For each pair of stimulants, the first stimulant was applied for 5 sec and then the second stimulant was applied for the same time. Recordings from a whole nerve preparation.

These results indicate that there are specific receptors for the three stimulants and that they are comprized almost exclusively in type 1 group of taste units. It is not known at present whether were observed between the inpulse numbers in the three stimulants share the same receptor or receptor sites although high correlation coefficients were observed between the impulse numbers in the first one or first two sec for each pair of the three stimulants: for example, 0.826, 0.911 and 0.745 for the first two sec, for uridine 5′-monophosphate vs. tryptophan, tryptophan vs. 50% sea water and 50% sea water vs. uridine 5′-monophosphate, respectively, based on 24 fibers which responded to all of the three stimulants. We have conducted a preliminary experiment on cross-adaptation among the three stimulants and obtained different results depending on combinations of the three stimulants. Quite unexpectedly, the response to tryptophan was almost completely depressed by the application of 50% sea water and vice versa. Whereas, the cross-adaptation between tryptophan and uridine 5′-monophosphate appeared to be only partial if any (Fig. 6). The results with tryptophan and 50% sea water suggest that the two share some common mechanism. However, they do not necessarily imply that they share the same receptors. While, the result with uridine 5′-monophosphate and tryptophan strongly suggests that the two have independent receptors in spite of the high correlation coefficient for the responses to them among the type 1 fibers. The fact that not all the betaine fibers were sensitive to proline and valine suggests that betaine receptors are different from those for proline or valine or that there are two kinds of betaine receptors, one sensitive also to proline and valine and the other betaine specific. The same argument can be applied to alanine fibers too, in relation to arginine sensitivity.

Specialistic responses to stimulants were also reported for fibers of the maxillary branch of the facial nerve in the puffer,[5] as mentioned above. In the puffer, uridine 5′-monophosphate sensitive fibers did not respond to amino acids. Unlike the present fibers, most of the amino acid sensitive fibers in the puffer responded to both of alanine and proline and there was a high correlation in the impulse frequencies for the two amino acids among these fibers. On the other hand, recent cross-adaptation experiments suggested that the two amino acids stimulate different receptors.* The latter observation is consistent with the present finding that there are independent fibers for the two amino acids in the yellowtail. Thus, in both species, alanine and proline seem to stimulate different receptors. In the palate of the Japanese eel also, it was reported that the response to proline recorded from the whole nerve was hardly affected by the application of alanine and vice versa.[7] Whereas, a severe depression of the response to proline was observed by applying alanine for 5 minutes and vice versa in the external receptors of the carp *Cyprinus carpio* innervated by the mandibular branch of the facial nerve and it was suggested that the two amino acids might share the same receptor mechainisms (transduction processes).[25] Independent receptors for alanine and arginine are also suggested in the channel catfish.[22] In the maxillary barbel nerve of this fish, two types of amino acid sensitive fibers have been observed. One is highly specific for arginine and the other is multi-sensitive and responds well to several amino acids including alanine. Cross-adaptation experiments with the whole nerve preparation also suggested independent receptors for alanine and arginine in this fish.

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