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Glossopharyngeal Taste Responses of the Channel Catfish to Binary Mixtures of Amino Acids

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Abstract

This study examines the neural processing of binary mixtures in the glossopharyngeal (IX) taste system of the channel catfish, *Ictalurus punctatus*, and finds that the nature of the components of a mixture determines the intensity of the neural response to it. Taste buds in fish innervated by IX are located along the gill rakers of the first gill arch and rostral floor of the oral cavity, and function primarily in the consummatory phase of feeding behavior; however, few studies of IX taste responses have been reported in any species of teleost. Here, we report IX taste responses to eight different binary mixtures of amino acids whose components were adjusted to be approximately equipotent in electrophysiological recordings. Four binary (group I) mixtures whose components were indicated from prior electrophysiological cross-adaptation experiments to bind to independent receptor sites resulted in significantly larger (22% average increase) integrated IX taste activity than four other (group II) binary mixtures whose components were indicated to bind to the same or highly cross-reactive receptor sites. These results are similar to those observed previously from facial nerve recordings in channel catfish, and to olfactory and taste responses in other vertebrate and invertebrate species. The group I results help to explain behavioral observations that chemical mixtures of chemosensory stimuli are often more stimulatory than their individual components.

Introduction

Studies of aquatic organisms clearly document that stimulus mixtures account for a major portion of the chemoresponsiveness to a natural extract or synthetic mixture (Carr, 1976; Carr and Chaney, 1976; Carr *et al.*, 1977; Adron and Mackie, 1978; Harada and Matsuda, 1984; Elliott, 1986). Previous studies of fish chemical senses were consistent in showing that mixtures of amino acids whose components were indicated to bind to relatively independent olfactory (Caprio and Byrd, 1984; Bruch and Rulli, 1988) and gustatory (Wegert and Caprio, 1991; Michel *et al.*, 1993) receptor sites resulted in enhanced electrophysiological chemosensory responses (Caprio *et al.*, 1989; Kang and Caprio, 1991; Kohbara and Caprio, 1996; Ogawa and Caprio, 1999). An 'enhanced' response is defined here and in the previous studies as a response to a binary mixture that is significantly greater than the response to a 50:50 mixture of the equipotent components. All previous electrophysiological recordings from the gustatory system of teleosts to binary mixtures were derived, however, solely from recordings from the facial nerve innervating taste buds located on the maxillary barbel of both freshwater (*Ictalurus punctatus*) (Ogawa and Caprio, 1999) and marine (*Arius felis*) (Kohbara and Caprio, 1996) catfishes. Extra-oral taste buds in catfish function primarily during appetitive feeding behavior, i.e. locating food at some distance from the animal (Bardach *et al.*, 1967; Atema, 1971; Valentincic and Caprio, 1994a). Although much has been learned concerning the physiology of the facial taste system in fish (Michel and Caprio, 1991; Marui and Caprio, 1992; Kohbara *et al.*, 1992), little is known concerning the responses of gustatory neurons innervating oral taste buds whose responses are critical during consummatory feeding behavior (Atema, 1971; Kanwal and Caprio, 1983; Valentincic and Caprio, 1994b). This report investigates the taste responses of the glossopharyngeal nerve in the channel catfish to binary mixtures of amino acids and compares the results with what is known concerning taste responses of the facial nerve to similar binary mixtures in the same species (Kohbara and Caprio, 1996; Ogawa and Caprio, 1999).

Materials and methods

Experimental animals

Fifteen channel catfish (*Ictalurus punctatus*), ranging from

40 to 80g body wt, were tested in the glossopharyngeal nerve experiments. The catfish were obtained from the Louisiana State University aquaculture center, kept in floating cages in a nearby university pond and fed regularly with commercial catfish chow. Catfish were transported as needed to the Animal Care Facility in the Life Sciences Building, maintained in a 250 l fiberglass aquarium in aerated, charcoal-filtered tap water (artesian well water) at 25°C and tested within 2 weeks of transfer.

Animal preparation

The catfish were immobilized with an i.m. injection of Flaxedil (gallamine triethiodide, 0.1 mg/100 g body wt; Davis and Geck Dept, American Cyanamid, Pearl River, NY) and wrapped in wet tissue paper. The catfish were placed on their right side and secured in a Plexiglas container onto a wax plate by metal clamps. A gill irrigation flow of ~500 ml/min of aerated, charcoal-filtered tap water contained the anesthetic, ethyl-*m*-aminobenzoate methane sulfonic acid (MS-222; 0.05%).

The gill irrigation water was perfused through the mouth and over the gills on the right side of the animal. The gill irrigation flow was prevented from contacting the gills within the surgical field on the left side. The IX nerve, innervating taste buds within the rostro-ventral oral epithelium and gill rakers of the first gill arch, was freed from surrounding tissue in mid- to ventral regions of the first gill arch and transected. A dam formed by muscle tissue was formed around a section of the peripheral cut end of the IX nerve to serve as a well for freshwater fish Ringer's to prevent desiccation of the exposed nerve. The IX nerve was teased into natural bundles since preliminary experiments indicated that a better signal:noise ratio of taste responses was obtained with small nerve bundles of IX rather than from whole nerve. Charcoal-filtered tap water (12 ml/min; pH 8.5–9.0, without MS-222) from a pressurized reservoir was maintained over the ventral oral epithelium. Additional doses of Flaxedil and MS-222 were provided as needed during the course of an experiment. Neural activity was recorded with a tungsten hook electrode, a.c. amplified, displayed on an oscilloscope, monitored aurally, integrated (0.5 s) and printed to a chart recorder.

Stimuli

Stock solutions of individual amino acids [L-alanine (Ala), L-arginine (Arg), glycine (Gly), L-monosodium glutamate (Glu), L-methionine (Met), L-proline (Pro), L-α-amino-β-guanidino-propionic acid (AGPA) (Sigma grade; Sigma Chemical Co., St Louis, MO)] were prepared weekly at 10 mM in charcoal-filtered tap water and were adjusted, if necessary, to pH 8.5. Amino acid solutions were stored in glass bottles at 4°C and were diluted to the desired experimental concentrations prior to testing. Stimulus solutions were added to a 0.5 ml Teflon® loop of a manual sample injection valve (model no. 1106; Omnifit USA, Atlantic Beach, NY) and injected into the water flow bathing the floor of the oral cavity. Inter-stimulus intervals were ≥3 min. A mixture of Ala, Arg and Pro (each at 3 mM) served as the search solution to locate a responsive IX nerve bundle since a previous report indicated that these three amino acids were highly potent to the IX taste system (Kanwal and Caprio, 1983). Photodensitometry studies indicated that the peak stimulus concentration delivered by the stimulus delivery system was ~75% of the applied concentration (Kohbara *et al.*, 1992). The reported stimulus concentrations were not corrected for dilution.

Experimental protocol and the mixture discrimination index (MDI)

Three sets of mixtures were tested: (I) four binary mixtures of amino acids whose components were indicated from prior electrophysiological cross-adaptation experiments of glossopharyngeal taste responses to bind to independent receptor sites (i.e. group I mixtures); (II) four binary mixtures whose components were indicated to bind to the same or highly cross-reactive receptor sites (i.e. group II mixtures) (Kanwal and Caprio, 1983); and (III) a control, pseudomixture composed only of L-alanine (i.e. L-Ala + L-Ala prepared with the identical protocol as the binary mixtures composed of different amino acids). The two component amino acids for each of the tested binary mixtures were tested separately and adjusted in concentration to provide approximately equal response magnitude based on the height of the stimulus-induced, phasic displacement of integrated multiunit activity. This standardization of the responses eliminated problems that could be due to differing ratios of Ala, Arg and Pro fiber types among the different IX nerve bundles tested (Ogawa and Caprio, 1994). This same procedure provided the standardization necessary to perform similar experiments on the facial taste system in this species (Ogawa and Caprio, 1999). Following standardization, the amino acid components across the respective binary mixtures were at concentrations between 10^{-6} and 10^{-3} M, well below those which result in a saturating response (Kanwal and Caprio, 1983); further, this concentration range for the amino acids is comparable to that expected to occur naturally within the vicinity of the fish during appetitive (lower concentrations) and consummatory (higher concentrations) phases of feeding behavior (Valentincic and Caprio, 1994a,b).

Responses to control water were baseline or <10% of phasic displacement from baseline compared with the response to the amino acid stimulus or to the binary mixture. Channel catfish taste responses recorded from the glosspharyngeal and vagal nerves (Kanwal and Caprio, 1983), as well as the facial nerve (Caprio, 1978), are phasic only (Figure 1). Each test series for a specific binary mixture and its components included the recording of taste responses: (i) to each of the two components tested individually at equipotent concentrations; (ii) to the binary

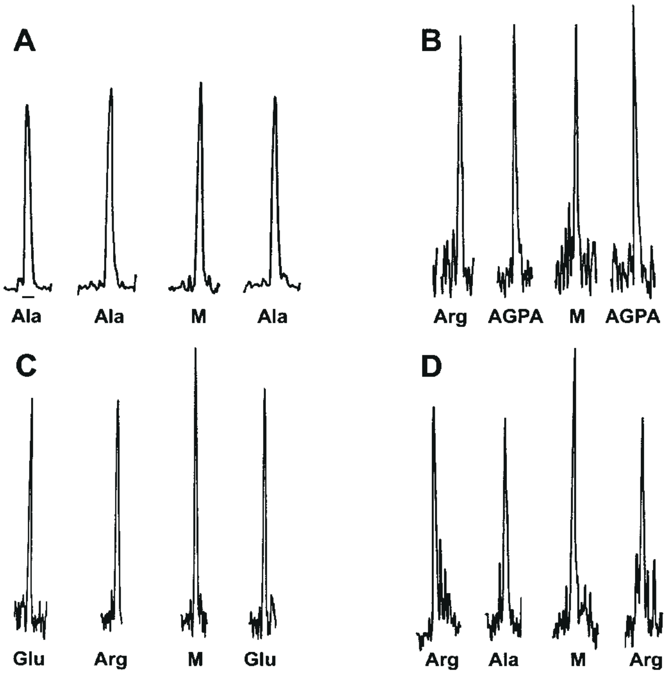

Figure 1 Representative integrated glossopharyngeal taste responses of the channel catfish to amino acids and to their binary mixtures. Responses to binary mixtures (M) and to their components: **(A)** control (10^{-5} M Ala + 10^{-5} M Ala); **(B)** group II (10^{-6} M Arg + 10^{-3} M AGPA); **(C)** group I (10^{-2} M Glu + 3×10^{-5} M Arg); and **(D)** group I (2×10^{-6} M Arg + 4×10^{-7} M Ala); all records were obtained from the same fish, but with different amplification. The line below the first response to Ala in (A) represents 12 s and is equivalent to the major portion of the response to each stimulus shown in (B–D).

mixture (tested twice); and (iii) to one of the components tested previously in (a) to confirm response reproducibility for this test series. The binary mixture itself was formed by mixing equal volumes of the two equipotent components. The response to (iii) was generally within ±10% of the magnitude of the response obtained to this component in (i). If the response to (iii) was ±20% or more of the magnitude of that obtained in (i), the results for the series were discarded. Subsequently, the MDI, defined as the averaged response to the mixture divided by the averaged response to the equipotent component stimuli, was calculated for each test series.

Data analysis

All MDI values for the different binary mixtures were analyzed by a randomized block analysis of variance (ANOVA, $\alpha = 0.05$); blocking on fish was fitted with Proc Mixed SAS (SAS Institute Inc., Cary, NC). Residuals were examined for normality using the Shapiro–Wilk test (Shapiro and Wilk, 1965) and shown to be consistent with a normal model. *A priori* contrasts were used to test for significance among the different binary mixtures.

Results

Performed were 61 experimental test series of binary mixtures comprising 305 taste responses and 12 control tests of the 'pseudomixture' (i.e. L-Ala + L-Ala) comprising 60 taste responses obtained from a total of 15 catfish. The experimental tests included 32 test series of four group I mixtures (Ala + Arg; Arg + Pro; Ala + Pro; Arg + Glu) and 29 test series of four group II mixtures (Ala + Met; Ala + Glu; Ala + Gly; Arg + AGPA) (Figures 1 and 2). A randomized block ANOVA of the data was highly significant ($P < 0.0001$; $F = 5.56$, df = 64). Post-hoc contrast tests indicated that MDIs for group I binary mixtures were significantly greater ($P < 0.0001$) than those for group II binary mixtures (Figure 1) and for controls ($P < 0.0001$) (Figures 1 and 2). There was no overlap in the 95% confidence intervals for the MDIs for group I (1.28–1.15; $n = 32$) and group II (1.06–0.96; $n = 29$) binary mixtures. No significant differences occurred in the MDIs for group II mixtures and controls ($P > 0.05$). There were also no significant differences ($P > 0.05$) between the MDIs among the group I binary mixtures or among the group II binary mixtures.

Discussion

Facial and glossopharyngeal taste fibers (i) innervate taste buds in extra-oral and oral regions, respectively; (ii) have different proportions and types of amino acid responsive taste fibers (Kohbara *et al.*, 1992; Ogawa and Caprio, 1994); (iii) terminate in different regions of the central nervous system (Morita and Finger, 1985; Kanwal and Caprio, 1987; Kanwal and Finger, 1992); and (iv) serve different functions in feeding behavior (Atema, 1971). Nonetheless, this study shows that both systems process binary mixtures in a similar manner. This study also indicates that glossopharyngeal taste responses of the channel catfish to binary mixtures of amino acids are similar to those of the facial taste nerve in both the channel (Ogawa and Caprio, 1999) and sea (Kohbara and Caprio, 1996) catfishes.

In previous studies (Ogawa and Caprio, 1999; Kohbara and Caprio, 1996) and in the present report, binary mixtures containing amino acids that did not readily cross-adapt (i.e. group I mixtures) resulted in enhanced neural activity compared with responses to binary mixtures whose components cross-adapted (i.e. group II mixtures) (Wegert and Caprio, 1991; Kanwal and Caprio, 1983; Michel *et al.*, 1993) (Table 1). MDIs calculated from both glossopharyngeal (present study) and facial (Ogawa and Caprio, 1999) taste recordings to group I binary mixtures indicate a similar degree of such enhancement of taste activity. Although a different proportion of the major amino acid fiber types occurs in facial (Kohbara *et al.*, 1992) and glossopharyngeal (Ogawa and Caprio, 1994) taste nerves in the channel catfish, the present results indicate that the degree of independence of the respective receptor sites for the majority of the component

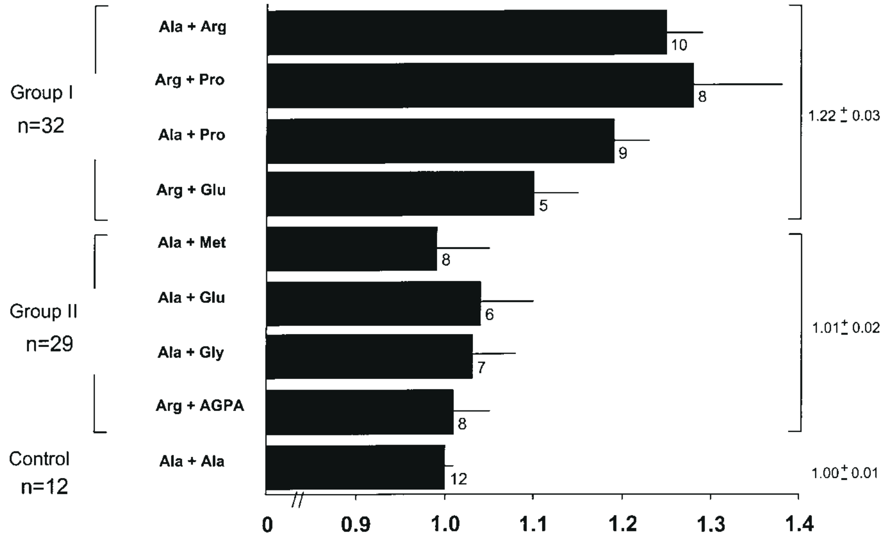

Figure 2 MDIs (mean ± SE) of integrated glossopharyngeal taste responses to binary mixtures of amino acids. Numbers adjacent to each bar indicate the number of fish tested with the respective binary mixtures (*n* = 15 total fish).

Table 1 Comparison of MDIs across different species and chemoreceptive systems

	I.p. taste IX[a] multifiber[b]	*I.p.* taste VII multifiber[c]	*I.p.* taste VII single fiber[c]	*A.f.* taste VII multifiber[d]	Hamster taste VII multifiber[e]	*I.p.* olfaction multiunit[f]	*I.p.* olfaction EOG[f]	Lobster olfaction single unit[g]
Group I[h]	1.22 ± 0.03 (32)[4]	1.16 ± 0.02 (44) [2]	1.17 ± 0.02 (116) [3]	1.41 ± 0.02 (107) [17]	1.30 ± 0.04 (?) [6]	1.58 ± 0.06 (28) [3]	1.43 ± 0.01 (238) [12]	1.49 ± 0.06 (31) [6]
Group II[i]	1.01 ± 0.02 (29)[4]	1.05 ± 0.02 (41) [2]	1.05 ± 0.02 (33) [3]	1.07 ± 0.01 (93) [9]	0.99 ± 0.04 (?) [4]	1.09 ± 0.05 (20) [3]	1.05 ± 0.01 (152) [10]	1.07 ± 0.01 (19) [5]

Values are means ± SE with numbers of tests in parentheses and numbers of different binary mixtures tested in brackets. EOG, electroolfactogram.

[a] Gustatory cranial nerve recorded (VII = facial; IX = glossopharyngeal).

[b] *Ictalurus punctatus* (freshwater channel catfish), present data.

[c] *Ictalurus punctatus* (freshwater channel catfish) (Ogawa and Caprio, 1999).

[d] *Arius felis* (sea catfish) (Kohbara and Caprio, 1996).

[e] Golden hamster (Hyman and Frank, 1980).

[f] *Ictalurus punctatus* (Caprio *et al.*, 1989).

[g] *Panulirus argus* (spiny lobster) (Cromarty and Derby, 1997).

[h] Binary mixtures whose components bind to relatively independent receptor sites.

[i] Binary mixtures whose components bind to the same and/or overlapping receptor sites.

stimuli (i.e. Ala, Arg, Pro) (Kanwal and Caprio, 1982; Wegert and Caprio, 1991) of the group I binary mixtures tested is comparable between the oral and extraoral taste systems.

The 22% average increase (i.e. an MDI of 1.22) in the glossopharyngeal taste response to group I mixtures compared with their components is equivalent to increasing the concentration of either component amino acid by 300–>400% as calculated from the dose–response function of the glossopharyngeal nerve to amino acid stimuli [$R = k(10)^{\log C/\gamma}$; γ = the number of log units of concentration required to obtain a 1 log unit change in response (Caprio, 1978) and is ~5 for potent amino acids except for L-proline, which is 3.4 (Kanwal and Caprio, 1983)]. Thus, any slight variation in stimulus concentration around the estimated 75% initial strength of the applied concentration across preparations would have minimal effect on any response difference obtained between group I and group II binary mixtures. These results parallel similar findings for the responses to binary mixtures of: (i) olfactory receptors in the channel catfish (Caprio *et al.*, 1989) (Table 1); (ii) olfactory receptors in the spiny lobster (Cromarty and Derby, 1997);

(iii) the facial taste nerve in the sea catfish (Kohbara and Caprio, 1996); and (iv) the facial (i.e. chorda tympani) taste nerve in the golden hamster (Hyman and Frank, 1980) (Table 1).

The response enhancement observed in the present multiunit preparations from the IX nerve to group I binary mixtures could have resulted solely from the simultaneous activation of different populations of taste fibers 'tuned' to the respective mixture components since taste fibers highly responsive to L-Ala, L-Arg and LD-Pro, respectively, have been identified in the IX nerve of the channel catfish (Ogawa and Caprio, 1994). However, since individual IX taste fibers can respond to amino acids other than their 'best' stimulus, the observed gustatory enhancement is more likely due to the simultaneous activation of relatively independent taste receptor sites that are expressed on the same and/or different taste cells innervated by a single taste fiber. A previous study of facial taste responses of channel catfish to group I binary mixtures of amino acids clearly showed that MDIs for single facial taste fibers were not significantly different from MDIs calculated from integrated multiunit preparations (Ogawa and Caprio, 1999) (Table 1); it is presumed to also be the case here for single IX taste fibers.

MDIs from the facial (Ogawa and Caprio, 1999) and glossopharyngeal (this report) nerves to group II binary mixtures did not show enhancement of taste activity (Table 1) and were not significantly different from controls. These results indicate that receptor sites for the component stimuli of the group II binary mixtures are the same or highly overlapping in both the oral and extra-oral taste systems of the channel catfish.

The results indicating that the binary mixture of L-glutamate and L-alanine acts as a group II mixture to taste cells innervated by the glossopharyngeal nerve confirm similar findings from recordings from the facial nerve in both the channel (Ogawa and Caprio, 1999) and sea (Kohbara and Caprio, 1996) catfishes. These results emphasize a major difference between the taste and smell of specific amino acids, as a binary mixture of L-glutamate and L-alanine acts as a group I mixture to the olfactory system and results in an enhanced olfactory receptor response (Caprio *et al.*, 1989).

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