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THE STIMULATION OF FILTER FEEDING IN THE PORCELAIN CRAB *PETROLISTHES CINCTIPES* RANDALL BY AMINO ACIDS AND SUGARS

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Abstract—1. The porcelain crab *Petrolisthes cinctipes* Randall is stimulated to filter feed by amino acids and sugars.

2. The most stimulatory amino acids are L-tyrosine > glycine > L-proline > L- and D-glumatic acid > γ-amino-*n*-butyric acid.

3. The most stimulatory sugars are trehalose and glucose.

4. Glutathione, peptides of glycine, and peptides of glycine and tyrosine are weakly stimulatory.

INTRODUCTION

There is a growing literature describing the electrophysiology of chemoreception and the anatomy of chemoreceptive structures in Crustacea. Hodgson (1958) recorded electrical activity from neurons of setae on the chelae and walking legs of crayfish using amino acids as stimuli. Case & Gwilliam (1961) and Case (1964) extended these findings by showing that chemoreceptors associated with dactyls of the walking legs of *Carcinus maenas*, *Cancer antennarius*, and *Cancer productus* respond to specific amino acids. More recently, Shelton & Laverack (1968) have specified certain setae on the dactyls of *C. maenas* and *Homarus vulgaris* as being chemoreceptive.

Little research has been reported on compounds promoting feeding behavior in Crustacea. Case *et al.* (1960) comment that amino acids evoking an electrophysiological response from dactyl chemoreceptors also arouse feeding movements when applied to the chelae and mouthparts of intact *C. maenas*. However, we have no knowledge of detailed experiments with Crustacea where feeding behavior is used as the criterion for chemoreception of specific compounds.

We observed that while the porcelain crab *Petrolisthes cinctipes* Randall will commence filter feeding when newly hatched brine shrimp *Artemia salina* are introduced into their aquarium, they are also stimulated to filter feed by the dialyzate of the hatch solution. As no macroscopic particles are present in the latter solution, the feeding must be stimulated by the chemoreception of small compounds. Since *P. cinctipes* is relatively abundant and reliably responds with stereotyped filter feeding, we set out to determine the specific compounds evoking the behavior.

MATERIALS AND METHODS

The porcelain crab *Petrolisthes cinctipes* Randall collected in the rocky high and middle tide zones of North Cove at Cape Arago, OR was used in this investigation. The animals were starved for at least 2 days prior to experiments by filtering the sea water entering the aquarium through a fiberglass trap. From a frequently replenished stock of about 200 animals, 5 randomly selected *P. cinctipes* were placed in each of 4 large finger bowls. The bowls contained 500 ml of filtered fresh sea water at 10–14°C with a pH ranging from 7.4–8.0. Small stones were introduced into the bowls to provide a natural substrate for the animals to grip; the room was darkened and the animals allowed to acclimate for 10–15 min.

A typical experimental run included a blank control, a glycine control, and 2 different compounds for assay. The blank control was 5 ml of distilled water gradually pipetted into one finger bowl. The glycine control was 5 ml of 10^{-1} M glycine in distilled water pipetted into another finger bowl. The latter served as a test of the feeding readiness of the crabs. Preliminary investigation indicated that this amino acid was particularly effective in promoting filter feeding, and if 60% of this control group did not initiate feeding, the results of the entire run were discounted. Into the third bowl was pipetted 5 ml of a 10^{-1} M solution of a compound to be assayed; this was repeated with the fourth compound in the remaining bowl. The animals were observed for 2 min, and those actively filter feeding were counted.

All solutions were made up immediately before use in distilled water to concns ranging from 10^{-1} to 10^{-4} M. For those compounds which are particularly acidic or basic, the sea water in the assay bowls was buffered using Tris to the pH of the blank and glycine control bowls.

Checks using fluorescein mixed with the glycine control showed that the pipetted material was initially rapidly distributed about the finger bowl aided chiefly by the current created by baling movements of the scaphognathites, and the beating of the flagella of the exopodites of the maxillipeds. Feeding was initiated best when there was a gradient or front approaching the animals. Preliminary experiments indicated that *Petrolisthes* would not feed if placed in finger bowls containing homogeneous solutions of glycine.

RESULTS

The filter feeding behavior of *Porcellana longicornis* has been carefully described by Nicol (1932) and that of *Petrolisthes cinctipes* is very similar. The porcelain crab firmly grips the substrate with the sharp dactyls of the 3 pairs of walking legs. The beating of the flagella of the exopodites on the maxillipeds aided

by the exhalent current of water from the branchial chamber draws particle laden water across the front of the carapace. Here the chemosensory antennules sample the water current for food. Upon chemical stimulation, the beating of the flagella stops and another water current created by the alternating movements of the third maxillipeds takes over. The 2nd through 5th segments of the third maxillipeds bear rows of long pinnate setae. When the limb is extended, the setae form a spoon-shaped scoop extending over a large area. The limbs are cast sideways alternately and flexed back towards the midline trapping suspended particles in the setae. A terminal brush of setae on the basal segment of the second maxilliped on the same side, scrapes the particles from the setae of the flexed maxilliped and passes them to the mouth (Nicol, 1932). The behavior is the same and continues for some minutes in *Petrolisthes* even though no particles are trapped in the setae of the third maxillipeds.

The effectiveness of aliphatic amino acids in promoting filter feeding was assayed in the first series of experiments (Table 1). Glycine was by far the strongest stimulant in this group. Concentrations of $<10^{-1}$ M evoked a feeding response in 76% of the crabs while concns of $<10^{-2}$ and $<10^{-3}$ M prompted 73% and 67% of the animals to feed. Only when the concn of glycine was $<10^{-4}$ M was the response appreciably reduced (17%). N, N-dimethyl glycine evoked little response, and N-methyl glycine suppressed feeding when compared to the blank control.

Table 1. Feeding activity evoked by aliphatic amino acids and related compounds

Compound	Concn (M)*	No. of animals feeding/ tested	Percentage feeding
control (distilled water)	—	41/360	11
control (glycine)	10^{-1}	273/360	76
glycine	10^{-2}	11/15	73
glycine	10^{-3}	20/30	67
glycine	10^{-4}	5/30	17
N-methyl glycine (sarcosine)	10^{-1}	0/15	0
N,N-dimethyl glycine	10^{-1}	1/15	7
DL-alanine	10^{-1}	6/15	40
DL-valine	10^{-1}	6/30	20
L-leucine	10^{-1}	6/15	40
L-isoleucine	10^{-1}	5/15	33
DL-serine	10^{-1}	8/15	53
DL-threonine	10^{-1}	8/15	53
taurine	10^{-1}	28/90	31
cysteine	10^{-1}	19/60	31
L-methionine	10^{-1}	25/45	56
L-methionine	10^{-2}	5/15	33
L-methionine	10^{-3}	1/15	7
DL-α-amino-*n*-butyric acid	10^{-1}	1/15	7
γ-amino-*n*-butyric acid	10^{-1}	8/15	53
γ-amino-*n*-butyric acid	10^{-2}	9/15	60
γ-amino-*n*-butyric acid	10^{-3}	4/15	27
L-lysine	10^{-1}	6/15	40
L-arginine	10^{-1}	6/30	20

* The concn noted is that of the introduced solution. The concn in the assay container after introduction of a 10^{-1} M solution is $<10^{-1}$–$10^{-3}>$ M; for a 10^{-2} M solution is $<10^{-2}$–$10^{-4}>$ M; for a 10^{-3} M solution is $<10^{-3}$–$10^{-5}>$ M; for 10^{-4} M solution is $<10^{-4}$–$10^{-6}>$ M.

Table 2. Feeding activity evoked by dicarboxylic, aromatic, heterocyclic amino acids, and related compounds

Compound	Concn (M)*	No. of animals feeding/ tested	Percentage feeding
control (distilled water)	—	36/285	13
control (glycine)	10^{-1}	206/285	72
L-aspartic acid	10^{-1}	1/15	7
DL-aspartic	10^{-1}	2/15	13
L-glutamic acid	10^{-1}	30/45	67
L-glutamic acid	10^{-2}	10/15	67
L-glutamic acid	10^{-3}	4/15	27
D-glutamic acid	10^{-1}	11/15	73
L-tyrosine	10^{-1}	15/15	100
L-tyrosine	10^{-2}	27/45	60
L-tyrosine	10^{-3}	28/45	62
L-tyrosine	10^{-4}	2/30	7
D-tyrosine	10^{-2}	3/15	20
D-tyrosine	10^{-3}	0/15	0
DL-phenylalanine	10^{-1}	5/15	33
L-β-3,4-dihydroxy-phenylalanine	10^{-1}	2/15	13
3-hydroxytyramine	10^{-1}	0/15	0
L-tryptophan	10^{-1}	18/30	60
L-tryptophan	10^{-2}	5/15	33
L-tryptophan	10^{-3}	3/15	20
L-proline	10^{-1}	25/30	83
L-proline	10^{-2}	10/15	67
L-proline	10^{-3}	6/15	40
L-proline	10^{-4}	1/15	7
hydroxy-L-proline	10^{-1}	3/15	20
L-histidine	10^{-1}	6/15	40

* See footnote of Table 1.

Gamma-amino-*n*-butyric acid, L-methionine, DL-serine, and DL-threonine were moderately stimulatory, prompting better than half the test animals to feed at $<10^{-1}$ and $<10^{-2}$ M concns. The other aliphatic amino acids were much less stimulatory than glycine, but were at least as stimulatory as the blank control. Eleven per cent of the crabs in the blank control container initiated feeding during the tests.

The dicarboxylic amino acid L-glutamic acid was strongly stimulatory (Table 2). Concentrations of $<10^{-1}$ and $<10^{-2}$ M caused 67% of the animals to commence filter feeding. Interestingly, the D-isomer of glutamic acid was also an effective stimulus since 73% of the crabs responded to a concn of $<10^{-1}$ M.

When the aromatic amino acid L-tyrosine at a concn of $<10^{-1}$ M was assayed, all of the test animals responded by filter feeding (Table 2). Concentrations of $<10^{-2}$ and $<10^{-3}$ M were not as stimulatory, but at least 60% of the animals fed. Only at $<10^{-4}$ M was the response at the blank control level. D-tyrosine at $<10^{-2}$ M and $<10^{-3}$ M was not an

Table 3. Feeding activity evoked by peptides

Compound	Concn (M)*	No. of animals feeding/ tested	Per-centage feeding
control (distilled water)	—	8/105	8
control (glycine)	10^{-1}	81/105	77
glycylglycine	10^{-1}	9/30	30
glycylglycylglycine	10^{-1}	0/15	0
glutathione	10^{-1}	2/30	7
glutathione	10^{-2}	2/15	13
L-tyrosylglycine	10^{-1}	4/10	40
glycyl-L-tyrosine	10^{-1}	2/15	13

* See footnote of Table 1.

effective stimulus. The structurally related compounds phenylalanine and L-β-3,4 dehydroxyphenylalanine (DOPA) were not effective stimuli, and 3-hydroxytyramine suppressed feeding. L-tryptophan was moderately stimulatory at $<10^{-1}$ M, but far less so at lower concns.

The heterocyclic amino acid L-proline was a particularly stimulatory compound (Table 2). Eighty-three per cent of the crabs responded to a concn of $<10^{-1}$ M, 67% to a concn of $<10^{-2}$ M, and 40% to $<10^{-3}$ M. Hydroxy-L-proline was only slightly more effective than the blank control in promoting feeding behavior.

Are peptides and tripeptides of the most stimulatory amino acids L-tyrosine and glycine effective stimuli? As seen in Table 3, glycylglycine is far less stimulatory then glycine (30% vs 77%), and glycylglycylglycine suppresses feeding. Glutathione (glutamylcysteineglycine) at $<10^{-1}$ and $<10^{-2}$ M is no more effective than the blank control. The peptides L-tyroslyglycine and glycyl-L-tyrosine are far less stimulatory than either glycine or tyrosine at the same concns.

The excretory end product urea promoted filter fedding by 40% of the crabs at a $<10^{-1}$ M concn. The degradation products trimethylamine oxide and glycine betaine were not particularly stimulatory (Table 4).

Since a variety of sugars have been identified in the hemolymph of crustacea and blood of other animals, several sugars were assayed to determine if they

Table 4. Feeding activity evoked by miscellaneous compounds

Compound	Concn (M)*	No. of animals feeding/ tested	Per-centage feeding
control (distilled water)	—	7/60	12
control (glycine)	10^{-1}	46/60	77
trimethylamine oxide	10^{-1}	2/15	13
glycine betaine	10^{-1}	4/15	27
urea	10^{-1}	6/15	40
acetate	10^{-1}	10/30	33
acetate	10^{-2}	0/15	0

* See footnote of Table 1.

Table 5. Feeding activity evoked by monosaccharides, disaccharides, amino sugars

Compound	Concn (M)*	No. of animals feeding/ tested	Per-centage feeding
control (distilled water)	—	13/180	7
control (glycine)	10^{-1}	128/180	72
α-D-glucose	10^{-1}	23/45	51
β-D-glucose	10^{-1}	21/45	47
D-galactose	10^{-1}	3/15	20
D-mannose	10^{-1}	2/15	13
β-D-fructose	10^{-1}	3/15	20
α-L-fucose	10^{-1}	2/15	13
D-xylose	10^{-1}	16/45	36
D-lyxose	10^{-1}	2/15	13
sucrose	10^{-1}	4/15	27
maltose	10^{-1}	9/45	20
trehalose	10^{-1}	18/30	60
trehalose	10^{-1}	3/15	20
trehalose	10^{-2}	2/15	20
glucosamine	10^{-3}	5/15	33
N-acetyl-D-glucosamine	10^{-1}	11/30	37

* See footnote of Table 1.

stimulate filter feeding in *Petrolisthes cinctipes*. All monosaccharides, disaccharides, and amino sugars tested were found to be more stimulatory than the blank control (Table 5). Of the monosaccharides, α-D-glucose and β-D-glucose at a concn of $<10^{-1}$ M prompted 51% and 47% of the animals to feed. Galactose was not a particularly strong stimulus as only 20% of the crabs initiated filter feeding at a $<10^{-1}$ M concn. D-xylose was mildly stimulatory.

Of the dissaccharides assayed, trehalose was most effective. A concn of $<10^{-1}$ M stimulated 60% of the crabs to feed, but lower concns were much less effective. The amino sugars glucosamine and N-acetyl-D-glucosamine were mildly stimulatory, prompting filter feeding in about one-third of the assay crabs.

DISCUSSION

The porcelain crab *Petrolisthes cinctipes* is stimulated to filter feed by amino acids and sugars. Of the compounds assayed, those most effective in initiating filter feeding are the amino acids L-tyrosine > glycine > L-proline > L- and D-glutamic acid > γ-amino-*n*-butyric acid, and the sugars trehalose > glucose. Thus, the stimulatory list includes representative aliphatic, dicarboxylic, heterocyclic, and aromatic amino acids, as well as a monosaccharide and disaccharide sugar. Case (1964) found that a far different spectrum of amino acids evoked neural activity when applied to the dactyl chemoreceptors of brachyuran species. DL-α-amino-*n*-butyric acid, taurine, L-glutamic acid, serine, DL-β-amino isobutyric acid, glycine betaine, and threonine were effective stimuli, while the sugars trehalose and glucose were non-stimulatory. However, he noted that peptides of glycine and other amino acids evoked less neural activity than the constituent amino acids and that proteins produced no activity. While it is not surprising that the filter-feeding anomuran species *P. cinctipes* is prompted to feed

by other amino acids, it is noteworthy that glycine peptides become less effective stimuli as the chain length is increased, and peptides containing tyrosine and glycine are only slightly stimulatory compared to those amino acids alone. Of those compounds very similar in structure, tyrosine is very stimulatory, but phenylalanine is weakly so; proline is stimulatory, but hydroxyproline is not; and glucose is mildly effective, galactose is not.

The essential amino acids required by *P. cinctipes* have not been determined. Among the Crustacea, the requirements of only 2 species of crayfish are known. Zandee (1966) and van Marrewijk & Zandee (1975) found the essential amino acids for *Astacus astacus* and *A. leptodactylus* to be valine, leucine, isoleucine, threonine, lysine, histidine, phenylalanine, and tyrosine, although the latter may be synthesized from dietary phenylalanine. These are in general the amino acids required by insects and vertebrates. If these same amino acids are essential to the diet of the porcelain crab, except for tyrosine, none is a strong filter feeding stimulus.

Filter feeding by *P. cinctipes* is strongly stimulated by the prominent free amino acids from muscle, nerve, and hemolymph of Crustacea as well as the most abundant free amino acids released by plankton. The free amino acids of muscles from a variety of marine crustacean species are highest in glycine, proline, arginine, and glutamate; from nerve, aspartate, glycine, taurine, and proline (Schoffeniels & Gilles, 1970). Hemolymph contains high concns of glycine, alanine, and proline (Jeuniaux, 1971). Hellebust (1965) determined the organic compounds excreted by 22 species of unicellular marine algae in culture. Considerable quantities of amino acids and peptides were released. The most abundant were proline, glutamic acid, as well as some arginine and aspartic acid. Webb & Johannes (1967) examined fresh zooplankton catches for the release of dissolved free amino acids. Whether the samples contained a diversity of copepod species, only *Calanus chilensis*, jellyfish and radiolarians, or chaetognaths and radiolarians, glycine was consistently the most abundant amino acid present, constituting an average of 31.7% of the total amino acids released. The major blood sugar of most animals is glucose, with trehalose found in low concns in the hemolymph of some species of marine Crustacea (Telford, 1968). These sugars stimulate filter feeding by *P. cinctipes*. The chemoreceptors of the porcelain crab are sensitive to the most abundant free amino acids and sugars from other species of Crustacea as well as plankton, and detection of those compounds, particularly glycine and proline, provides the chemical clue of food availability.

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