Behavioral study of chemoreception in the sea star *Marthasterias glacialis*: Structure-activity relationships of lactic acid, amino acids, and acetylcholine

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**Summary.** 1. Behavioral responses of *Marthasterias glacialis* to low molecular compounds were studied under laboratory conditions. Feeding postures, stomach eversions and locomotion of initially inactive animals can be released with very dilute solutions of lactic acid, neutral 2 and 3 carbon amino acids, L isomers of 4 to 6 carbon neutral amino acids, L-arginine, acetylcholine iodide, and several of their analogues.

2. Hunger was induced by temporary withdrawal of food. Responsiveness to feeding stimuli was controlled with L-cysteine and L-leucine.

3. The lowest behavioral thresholds for the most effective feeding stimuli were $3 \times 10^{-11}$ mol/l for both enantiomers of lactic acid, $10^{-8}$ mol/l for L-proline and both enantiomers of cysteine and $10^{-7}$ mol/l for acetylcholine iodide and some of the effective neutral amino acids.

4. The behavioral threshold values for chemical stimuli differed by a factor between 30 and 100 in different sea stars. The test concentration was $3 \times 10^{-7}$ mol/l, the level at which L-cysteine elicited a complete feeding response from all the animals. Structure-activity comparison of substances less effective than the control stimulus was thus possible. The behavioral threshold of fully effective substances was determined later.

5. The independence of receptor mechanisms for different substances can be inferred as:

- L-cysteine controlled responsiveness is not always accompanied by responsiveness to neutral amino acids.
- Autotomized *Marthasterias* arms crawled after stimulation with lactic acid, cysteine, and acetylcholine iodide but did not respond to the feeding stimuli betaine and L-proline.
- An animal became inactive if electric shocks were paired with L-proline or L-cysteine emanating from an ‘electric’ food model. It was subsequently easier to release feeding with a substance which had not been presented with shocks.

6. $\alpha$-carboxyl and $\alpha$-hydroxyl groups are prerequisites for the attachment of lactic and glycolic acids to the receptor subsites. The methyl group of lactic acid contributes to its effectiveness.

7. The $\alpha$-amino and ionised $\alpha$-carboxyl groups were necessary components of neutral amino acids which stimulated the animals. L- and D-enantiomers of alanine were equally effective. Responsiveness to neutral amino acids with 4 to 6 carbons was stereoselective.

8. Cysteine and some of its analogues interact with two different receptor mechanisms. The sulfhydryl group releases vigorous searching for food. The crawling and the feeding postures are triggered by thioglycolic acid, L-cysteine methylester HCl, Na$_2$S, and reduced glutathione. These substances prevent the animals from discriminating between sea water soaked and test food models. Substances with $\alpha$-carboxyl, $\alpha$-amino, and sulfhydryl groups such as L- and D-cysteine, DL-homocysteine, and N-acetyl-L-cysteine release the complete sequence of feeding motions and enable the selection of flavoured food models. It is postulated that behavior is influenced via the neutral amino acid receptors.

9. Free dissolved acetylcholine iodide, acetyl-$\beta$-methyl-choline, and cholinemethylsulphate are effective feeding stimuli for *Marthasterias glacialis*.

**Introduction**

Evidence for echinoderm chemosensory abilities was derived from simple behavioral experiments. The absence of obvious chemoreceptor organs and our ignorance of the function of the nervous sys-
tem in echinoderms make electrophysiological studies difficult. Olfactory structures are situated on the distal tube feet of *Marthasterias glacialis* and other sea stars (Prüho 1890). Echinoderms obtain sensory input from the epithelia (Cobb 1970). The microvilli of the asteroid epithelia are embedded in acid mucopolysaccharide and acid mucoid ‘cuticle’ layers (Santos and Sasso 1970; Engster and Brown 1972; Perpeet and Jangoux 1973). At least some of the microvillar cells and the ciliated epithelial cells have processes which extend into the basi-epithelial plexus (Kawaguti and Kamishima 1964; Coleman 1969; Cobb 1970; Weber and Grosmann 1977). Structures similar to the ophiurid ‘Stülchen’ (Whitfield and Emson 1983) are unknown in asteroids.

Behavioral studies of chemosensitivity in echinoderms have been on asteroids and ophiurids. Stomach eversion can be induced by acetylcholine in *Asterias forbesi* (Anderson 1954). Urea, lactic acid, and succinic acid induce *Asterias vulgaris* to approach the stimulus source (Zafiriou 1972). L-glutamic acid and a mixture of amino acids produce a change from a positive to a negative rheotactic behavior in *Asterias rubens* (Casilli 1972). L-cysteine, L-proline, some neutral amino acids, acetylcholine iodide, and their analogues release the entire sequence of feeding motions: orienting response, searching for and finding food, feeding posture, and stomach eversion in *Marthasterias glacialis* (Valentinčič 1973, 1975, 1980a, b). Betaine and some neutral amino acids induce prey following in the crown-of-thorns starfish *Acanthaster planci* (Moore and Huxley 1976). Some authors claim that high molecular weight compounds are involved in asteroid food searching (Heeb 1973; Collins 1975). Responses to stimulating chemicals depend on motivation. Habituation to feeding stimuli or sensitisation were not observed under test conditions (Valentinčič 1983).

The extreme sensitivity of echinoderms to chemical stimuli was reported for the brittle star *Ophiura ophiura* (Moore 1984). It can detect amino acids and lactic acid at concentrations as low as $10^{-10}$ to $10^{-11}$ mol/l, as shown by electrophysiological recordings from the radial nerve cord.

Structure-activity relationships of hypothetical chemoreceptor sites of *Marthasterias glacialis* are described. The behavioral techniques used in the experiments presented must suffice until the development of electrophysiological techniques for recording from the animal’s unusual segmental nervous system with no through conducting fibers. The short nerve fibers are 0.1 to 0.8 μm in diameter.