6-1988

Chemoreceptors Regulate Discharge of Microbasic p-mastigophores in the Sea Anemone, AIPTASIA PALLIDA

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Abstract

CHEMORECEPTORS REGULATE DISCHARGE OF MICROBASIC p-MASTIGOPHORES IN THE SEA ANEMONE, Aiptasia pallida

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Recently, using cnida-mediated measurements of adhesive force, Thorington and Hessinger (1984, 1988a, b) identified two different chemoreceptors involved in triggering cnida discharge on the tentacles of the sea anemone, Aiptasia pallida. These two classes of receptors were shown to interact with glycine and N-acetylneuraminic acid (NANA). We now show that the discharge of the microbasic p-mastigophores, one of the three types of cnidae present on the tentacles of A. pallida, is under the controlling influence of these two classes of cnidocyte-associated chemoreceptors. We also demonstrate that the number of discharged microbasic p-mastigophores adhering to the test probes is proportional to and linearly related to a component of the measured adhesive force. Our findings, thus, validate using measurements of adhesive force as indicators of the relative extents of cnida discharge. Furthermore, we calculate that the contribution to adhesive force made by a single mastigophore is approximately 0.17 mgf.

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A Thesis in Partial Fulfillment of the Requirements

for the Degree Master of Arts

in Biology

June 1988

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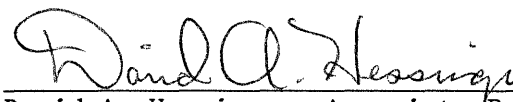

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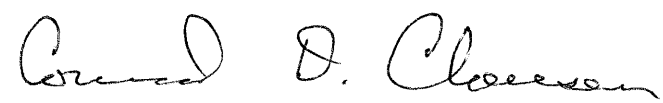

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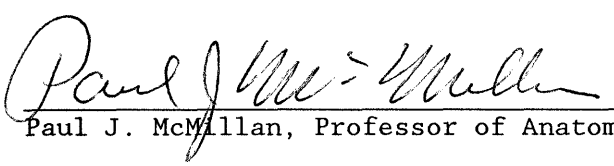

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ACKNOWLEDGEMENTS

We thank Drs. G. Watson, and P. McMillan for their advice in support of this project. Funded in part by BRSG grant RR 05352-24 and NSF grant DCB-8609859 to Dr. D. A. Hessinger.

INTRODUCTION

Feeding in cnidarians consists of prey capture, the feeding response (coordinated writhing of tentacles and opening of the mouth), and ingestion. As far back as 1892 (Nagel) it was suggested that the substances associated with food can elicit feeding. More recently prey-derived substances have been identified which activate the feeding responses in several species (Loomis, 1955; Lenhoff, 1968; Lenhoff and Heagy, 1977). In addition, Thorington and Hessinger (1984, 1988a, b), measuring cnida-mediated adhesion of tentacles, have identified and characterized at least two different chemoreceptors involved in triggering cnidae to discharge in the sea anemone, Aiptasia pallida.

In this paper we demonstrate that the two chemoreceptor systems identified by Thorington and Hessinger (1984, 1988b) regulate the discharge of microbasic p-mastigophores, the major type of penetrant cnida found in the tentacles of A. pallida. In addition, we show that cnida-mediated adhesive force measurements correlate with the numbers of mastigophores induced to discharge.

MATERIALS AND METHODS

Large numbers of sea anemones (A. pallida, Miami strain) were cloned in glass trays containing natural seawater and fed daily with Artemia nauplii (Hessinger and Hessinger, 1981). Forty animals of similar size were selected and placed in separate finger bowls. These anemones were maintained under controlled conditions consisting of a 12:12 photoperiod with ambient temperatures of 24 ± 1°C. The seawater was changed daily. Animals to be used in experiments were starved for 72 h.

Two different, known chemosensitizing agents glycine and N-acetylneuraminic acid (NANA) (Thorington and Hessinger, 1984, 1988a, b) were tested for their ability to effect discharge of tentacle mastigophores. The seawater in the finger bowls was replaced with solutions containing one of these agents at various concentrations and the anemones were allowed to adapt for 10 min. before cnidocyte responsiveness was measured. Cnidocyte responsiveness to combined chemical and tactile stimulation was determined both by measuring cnida-mediated adhesive force and by microscopically counting discharged mastigophores which had adhered to the adhesive force test probes.

Adhesive force was measured using test probes consisting of insect pins having nylon heads 0.8 ± 0.01 mm in diameter (Thorington and Hessinger, 1988b). The pin heads were coated with approximately 0.06 mm of 30% (w/v) gelatin and stored at 4°C in a humidified container until used within 24 h. To measure adhesive force the test probes were attached to a force transducer (Model FT-03, Grass Instr.) and a strip-chart recorder (Thorington and Hessinger, 1988a). The transducer was calibrated with gravitometric weights and adhesive force measurements

were expressed in hybrid units of mg-force (mgf). Each bowl, with a single sea anemone in seawater containing the appropriate concentration of chemosensitizer, was rinsed by hand until the test probe touched just behind the apical tip of a tentacle. After contact the bowl was lowered slowly until the tentacle and the coated pinhead separated. The force necessary to separate the probe from the tentacle was recorded. The underlying assumptions of this measurement are that the measured adhesive force is mediated by discharged cnidae, such as the penetrant mastigophore nematocysts and the adherent spirocysts, and that the magnitude of adherent force is proportional to the number of cnidae discharged (Thorington and Hessinger, 1988a, b).

Probes used to measure adhesive force were also used to quantitate the mastigophores adhering to them. Probes were placed in individual microtiter wells (Microtest 11, Falcon Plastics) with 40 ul of an enzyme and detergent solution (1% Trizyme, Amway Products). The solution was prepared in distilled water and clarified by centrifugation at 2,000 x g for 30 min and then frozen in 1.5 ml aliquots until used. Probes were incubated in Trizyme for 4 h at room temperature to hydrolyse the gelatin and release from the probe the mastigophores and other types of cnidae which are protease resistant (Blanquet and Lenhoff, 1966). Probes were then removed and the number of mastigophores remaining in each well enumerated with the aid of an inverted light microscope at 200X.

Separate animals were tested at each concentration of sensitizer. Five to ten probes (one per tentacle) were used on each animal to deter-

mine both adhesive force and the number of discharged mastigophores. Daily experimental means were calculated from these measurements on four different days. Replicate experiments were performed on four different days for both glycine and NANA. Each data point expressed in the figures represents the mean of the daily experimental means (n=4). Range bars indicate the standard error of the mean (95% confidence limits). Linear regression analysis and determination of sensitizers concentrations that produce a half-maximum response ($K_{0.5}$) was performed with the aid of a computer-assisted graphics and data formatting program (Dorgan and Hessinger, 1984).

RESULTS

The dose-response curves of mean adhesive force measurements at different concentrations of glycine (Fig. 1, triangles) and of the mean number of discharged mastigophores adherent to the test probe (Fig. 1, circles) are both biphasic. These two dose-response curves coincide somewhat, with each having a broad area of sensitization at lower concentrations of glycine, a maximum response (Emax) at about 10^{-6}M glycine, and a broad region of apparent desensitization at higher concentrations. Several differences are also noted. Unlike the adhesive force measurement which initially drops steeply at 10^{-9}M glycine before rising to its maximum effect, the number of mastigophores rises steadily to a maximum value. In addition, the maximum adhesive force only increases approximately 15% over control values (i.e. in the absence of glycine), while the maximum number of adherent mastigophores increases more than two-fold over control values. Furthermore, the concentrations of glycine at which the half-maximum response occur ($K_{0.5}$) are somewhat different, being 5.0×10^{-8}M for adhesive force measurements and 1.6×10^{-7}M for the number of adherent mastigophores.

The effects of different concentrations of NANA on the mean adhesive force measurements (Fig. 2, triangles) and on the mean number of adherent mastigophores (Fig. 2, circles) are also biphasic and essentially coincidental. Both dose-response curves have areas of sensitization of low concentrations of NANA, Emax values at 10^{-5}M NANA, and a region of apparent desensitization at higher concentrations. On the other hand, the adherent force measurement shows only at 25% increase in the number of adherent mastigophores from control values to the maximum response, whereas the maximum number of adherent mastigophores increases

nearly three-fold. In addition, the concentrations of NANA at which half-maximal responses ($K_{0.5}$) occur are 3.2×10^{-7}M for adhesive force and 7.8×10^{-8}M for the number of adherent mastigophores.

The mean number of discharged mastigophores adhering to test probes is directly proportional to the mean adhesive force measured by the probes when stimulatory concentrations of glycine and NANA (0 to 10^{-6}M and 0 to 10^{-5}M, respectively) are used (Fig. 3). The extrapolated, least-squares, fitted line for these data intercepts the abscissa to the right of the origin and indicates that the tentacles have an inherent adhesiveness of approximately 43.3 mgf that is apparently unrelated to the discharge of mastigophore nematocysts. When the inherent adhesiveness of the tentacle is subtracted from each mean measurement of adhesive force and expressed as the mean, corrected adhesive force per mastigophore, we find that the contribution of each mastigophore to the adhesive force measurement is independent of the number of mastigophores discharged (Fig. 3, insert). At higher, desensitizing concentrations of sensitizer, however, the number of discharged mastigophores does not correlate with measured adhesive force (data not shown).

DISCUSSION

The adhesion of tentacles to test objects has been used to qualitatively estimate the extent of *in situ* cnida discharge (Williams, 1968; Lubbock, 1979). More recently, using a novel technique to quantify cnida-mediated adhesion, Thorington and Hessinger (1984, 1988a, b) identified two classes of specific chemoreceptors involved in triggering cnidae to discharge. All adhesion methods assume that the adhesion between test objects and tentacles is mediated by discharged cnidae and that the adhesive force between the test object and the stimulated tentacle is directly proportional to the number of cnidae discharged. This underlying assumption has never been experimentally validated.

In sea anemones the cnidae of the tentacle include the adherent spirocysts and the penetrant nematocysts. In acontiate sea anemones, such as *A. pallida* and *Haliplanella luciae*, the cnidae present include the spirocysts, the microbasic p-mastigophores, and the basitrichous isorhizas (Hand, 1955). Of these three types of cnidae the basitrichous isorhizas comprise a comparatively small portion of the total cnidae present in the tentacles of *A. pallida* (Giebel, unpublished observations) and *H. luciae* (Watson and Mariscal, 1983) and are unlikely to contribute significantly to the adhesive force measurements. In addition to contributions from discharged cnidae, the mucous on the tentacle surface contributes approximately 30 mgf, in the absence of cnidae discharge, to the measure of tentacle adhesive force (Thorington and Hessinger, in preparation). Until this present paper, however, no one has shown that measures of tentacle adhesive force are directly proportional to the number of specific cnidae discharged into the test object.

We now show, under conditions of defined chemosensitization, that adhesive force measurements correlate linearly and directly with the number of discharged mastigophores adhering to the test probes (Fig. 3). We also calculate the contribution of individual mastigophores to adhesive force measurements. In addition, we show that the discharge of mastigophores is dose-responsive to known chemosensitizing agents.

Adhesive force measurements represent the sum of all participating adhesion factors, including cnida-mediated and non-cnida-mediated (i.e. "stickiness") factors. The data in this paper show that under conditions of defined chemosensitization a component of adhesive force correlates directly with the number of adhering mastigophores while another component of the adhesive force measurement is independent of the adhering mastigophores (Fig. 3).

At stimulatory doses of glycine and NANA the increments of adhesive force greater than 43 mgf correlate linearly with the number of adhering mastigophores (Fig. 3). By extrapolating this linear correlation to the adhesive force expected in the absence of discharged mastigophores (Fig. 3) we determine that adhesive force measurements can be divided into contributions that are mastigophore-dependent and mastigophore-independent. Contributions to adhesive force up to 43 mgf are independent of mastigophores and due to, most probably, a combination of contributions, such as discharged spirocysts and the inherent "stickiness" of the surface mucous. Contributory increments in excess of 43 mgf are due to discharged mastigophores. From the slope of the linear correlation between discharged nematocysts and adhesive force we calculate that the

contribution of each discharged mastigophore to adhesive force is approximately 0.17 mgf. The same approximate value is obtained as the ordinate intercept of a plot when 43 mgf is subtracted from the adhesive force measurements and then plotted as mgf/mastigophore versus the number of adherent mastigophores (Fig. 3, insert).

The observed correlation between number of adhering mastigophores and adhesive force occurs only at stimulatory doses of the tested sensitizing agents. At higher, desensitizing doses of sensitizers the adhesive force measurements do not correlate very well with the number of adhering nematocysts possibly indicating that changes in other contributions to adhesive force, such as from discharged spirocysts, are also occurring. Discharged spirocysts are difficult to accurately count with the light microscope, however and most likely other means of measuring the extent of spirocyst discharge will have to be developed.

Williams (1968), using the sea anemone Halliplanella luciae, concluded that only spirocysts and not mastigophores were chemosensitized by food extracts. We find that the mastigophores of A. pallida are chemosensitized by optimum concentrations of both glycine and NANA (Figs. 1 and 2, circles). Similar findings have been found by us for H. luciae (Watson and Hessinger, in preparation).

The influence of different concentrations of glycine and NANA on the number of adherent mastigophores produces biphasic dose-response curves similar to those obtained from measuring adhesive force under the same conditions (Figs. 1 and 2, circles). This suggests that glycine and NANA effect both the measurements of adhesive force and the number of adherent mastigophores via related mechanisms. This suggestion is

also supported by the already noted correlation between the number of mastigophores discharged and the measured adhesive force (Fig. 3). Thus, we conclude that adhesive force measurements are a quantitative indicator of the number of penetrant nematocysts discharged from chemosensitized sea anemone tentacles.

The major difference between the dose-response curves for adhesive force and for the number of discharged mastigophores is that there is proportionally a greater increase in the number of adherent mastigophores at Emax values than in adhesive force. For example, the maximum increase in number of discharged mastigophores is two- and three-fold for glycine and NANA, respectively, as compared to only 15% and 25% increases in adhesive force, respectively. The differences between these increases can be explained in terms of adhesive force being a composite measure of several contributing factors, including discharged nematocysts, as previously discussed in detail. Since the number of discharged mastigophores is only one of at least three contributing factors it is possible that a two- to three-fold change in number of discharged mastigophores could cause only a 15% to 25% increase in adhesive force.

In summary, we have shown that the number of penetrant nematocysts adhering to test probes is proportional to the measured adhesive force. This validates the use of adhesive force measurements to indicate the relative extent of total cnida discharge. In addition, we have also shown that the discharge of microbasic p-mastigophore nematocysts is under the controlling influence of at least two classes of cnidocyte-

associated chemoreceptor systems that are responsive to glycine, on the one hand, and to NANA, on the other.

FIGURE LEGENDS

Figure 1. The effects of glycine on adhesive force (mgf) and on the number of discharged mastigophores adherent to test probes. Values for adhesive force (triangles) and for the number of discharged mastigophores (circles) are expressed as means of the daily means of separate measurements with the vertical bars representing standard errors.

Figure 2. The effects of N-acetylneuraminic acid (NANA) on adhesive force (mgf) and on the number of discharged mastigophores adherent to test probes. Values for adhesive force (triangles) and for the number of discharged mastigophores (circles) are expressed as means of the daily means of separate measurements with vertical bars representing standard errors.

Fig. 3. Correlation of the number of adherent discharged mastigophores to measured adhesive force (mgf). Horizontal and vertical bars represent standard errors of the mean (95% confidence limits) for adhesive force and for the number of adherent mastigophores, respectively. Plotted values represent all data measured at stimulatory concentrations of glycine (open circles) and NANA (closed circles) from Figs. 1 and 2. Insert. Plotted values obtained by subtracting 43.3 mgf from each mean measurement of adhesive force and dividing by the number of adherent mastigophores, then graphically expressing these values as a function of the number of adherent mastigophores.

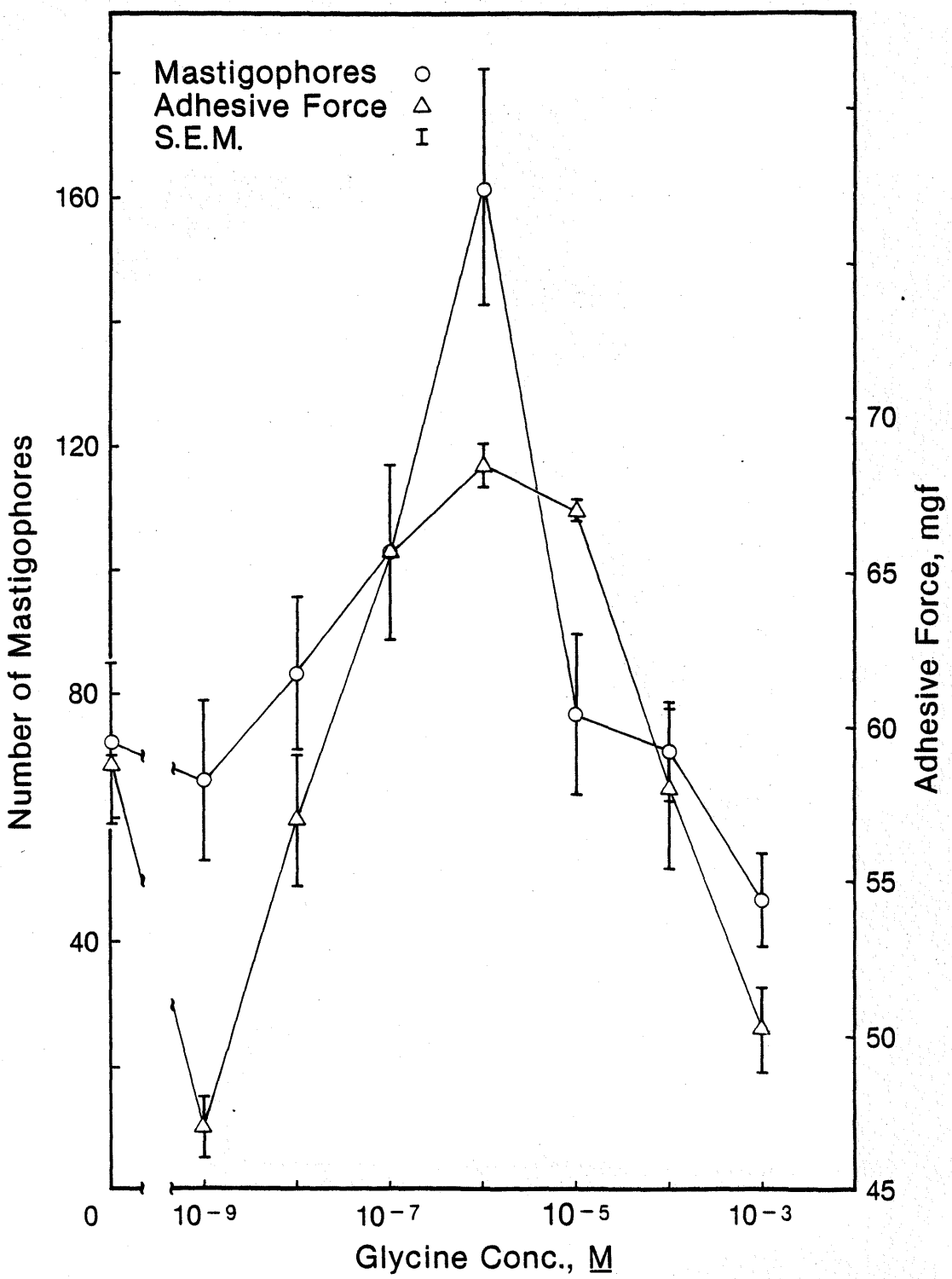
Mastigophores
Adhesive Force
S.E.M.
Number of Mastigophores
Adhesive Force, mgf
160
120
80
40
0
70
65
60
55
50
45
10^{-9}
10^{-7}
10^{-5}
10^{-3}
Glycine Conc., M

Figure 1.

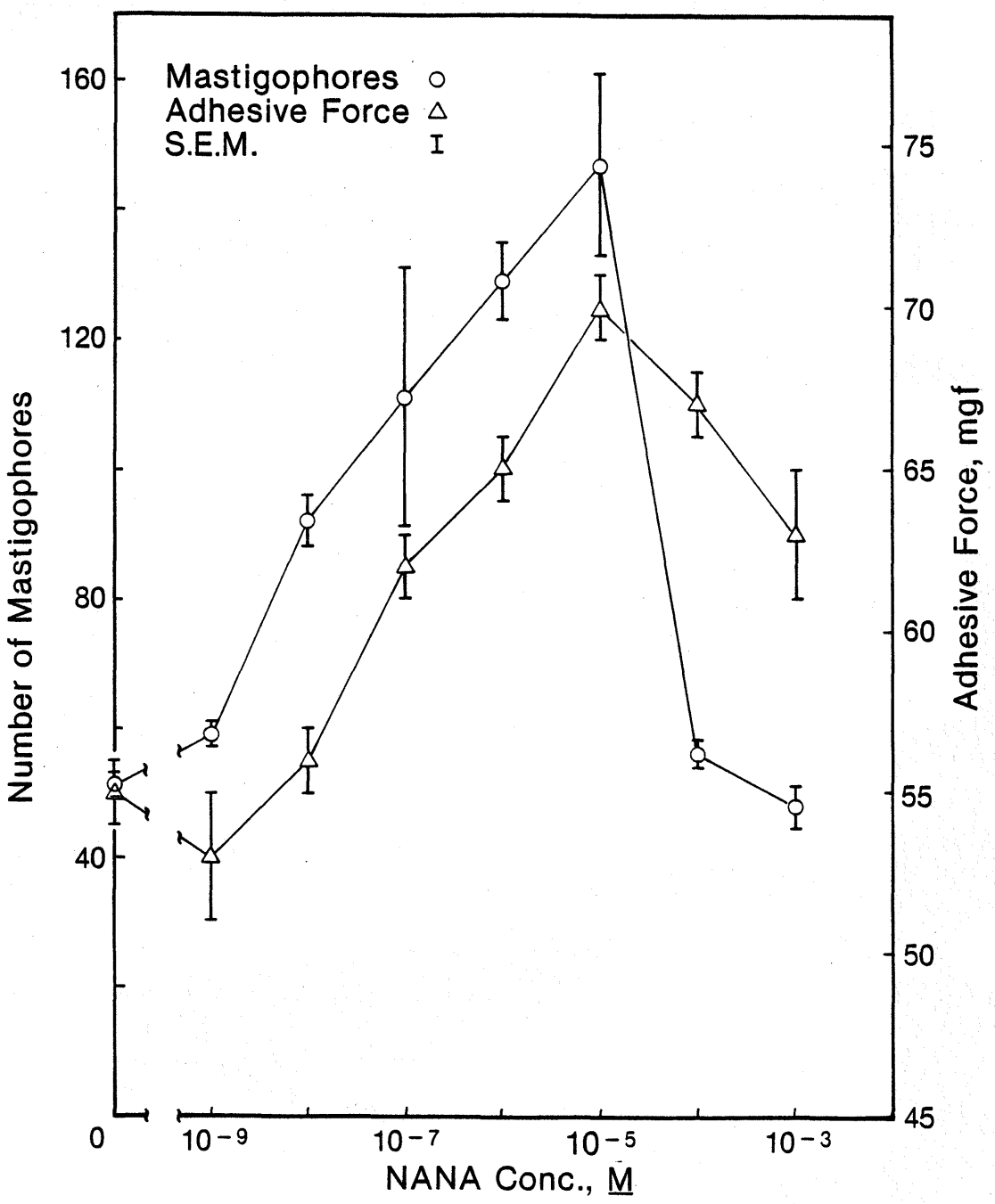
Mastigophores
Adhesive Force
S.E.M.
160
120
80
40
0
75
70
65
60
55
50
45
Number of Mastigophores
Adhesive Force, mgf
10⁻⁹
10⁻⁷
10⁻⁵
10⁻³
NANA Conc., M

Figure 2.

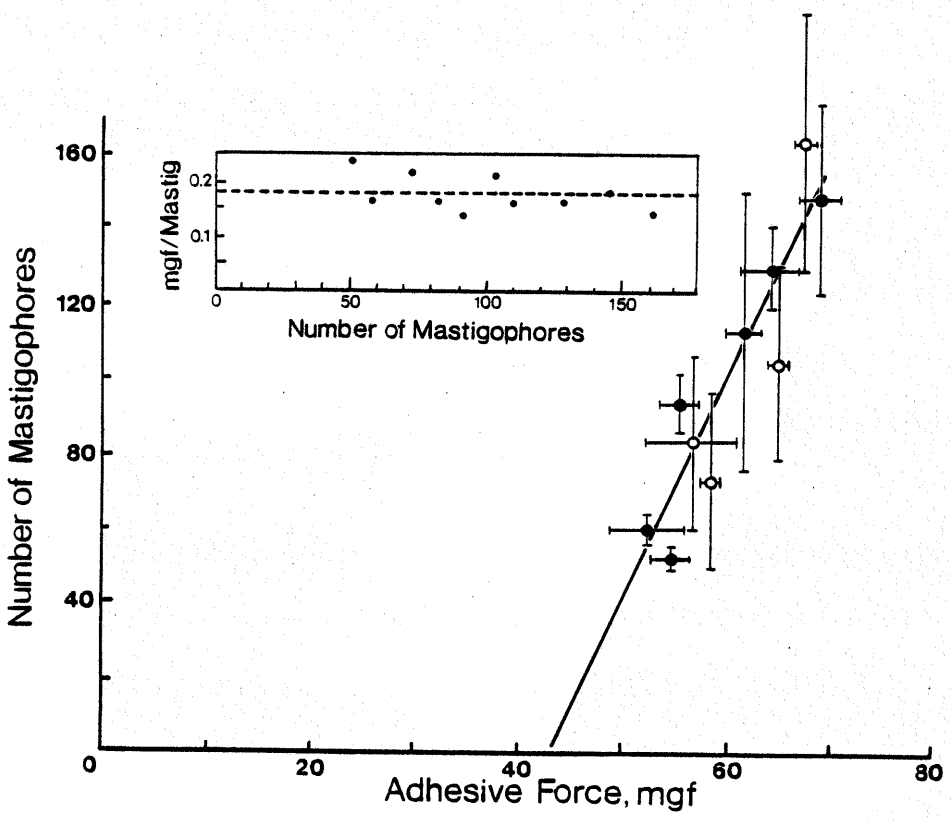
Number of Mastigophores
160
120
80
40
0
20
40
60
80
Adhesive Force, mgf
mgf/Mastig
0.2
0.1
0
50
100
150
Number of Mastigophores

Figure 3.

LITERATURE CITED

BLANQUET, R. AND H. M. LENHOFF. 1966. A disulfide-linked collagenous protein of nematocysts capsules. Science 154: 152-153.

DORGAN, L. AND D. A. HESSINGER. 1984. GRAFPAC, a graphics and format package for the Apple II and (IIe) computer. Copyright 1984.

HAND, C. 1955. The Sea Anemones of Central California. Part 3. The acontiarian anemones. Wasmann J. Biol. 13: 189-251.

HESSINGER, D. A. AND J. A. HESSINGER. 1981. Methods for rearing sea anemones in the laboratory. Pp. 153-179, in Committee on Marine Invertebrates. National Academy Press, Washington, D. C.

LENHOFF, H. M. 1968. Chemical perspectives on the feeding response, digestion, and nutrition of selected coelenterates. Pp. 157-221 in Chemical Zoology. B. Scheer and M. Florkin, eds., Academic Press, New York.

LENHOFF, H. M. AND W. HEAGY. 1977. Aquatic invertebrate model systems for study of receptor activation and evolution of receptor proteins. Ann. Rev. Pharmacol. Toxicol. 17: 243-258.

LOOMIS, W. F. 1955. Glutathione control of the specific feeding reactions of Hydra. Ann. N. Y. Acad. Sci. 62: 209-228.

LUBBOCK, R. 1979. Chemical recognition and nematocyte excitation in a sea anemone. J. Exp. Biol. 83: 283-292.

NAGEL, W. 1892. Das geschmacksinn der actinien. Zool. Anz. 15: 334-338.

THORINGTON, G. U. AND D. A. HESSINGER. 1984. Identification and partial characterization of cnidocyte chemoreceptors on the sea anemone, Aiptasia pallida. J. Cell Biol. 99(4): 221a.

THORINGTON, G. U. AND D. A. HESSINGER. 1988a. Identification of chemoreceptors involved in triggering cnida discharge in a sea anemone. Bio. Bull., in press.

THORINGTON, G. U. AND D. A. HESSINGER. 1988b. In Biology of Hydra. D. A. Hessinger and H. N. Lenhoff, eds., Academic Press, Orlando, Fl., in press.

WATSON, G. M. AND R. N. MARISCAL. 1983. Comparative ultrastructure of catch tentacles and feeding tentacles in the sea anemone Haliplanella. Tissue and Cell 15(6): 939-953.

WILLIAMS, R. B. 1968. Control of the discharge of cnidae in Diadumene luciae (Verrill). Nature 219: 959.