

Control of Cnida Discharge: II. Microbasic p-Mastigophore Nematocysts are Regulated by Two Classes of Chemoreceptors

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Abstract. Using tentacles of the sea anemone, *Aiptasia pallida*, Thorington and Hessinger (1984, 1988a, b) recently identified two classes of chemoreceptors involved in sensitizing cnidocytes to discharge cnidae in response to mechanical stimuli. Discharge of cnidae was quantified by measuring adhesive force between the tentacles and a test object. This measurement is assumed to reflect the contribution of the three types of cnidae present in the tentacles of *A. pallida*: the adherent spirocysts and two types of penetrant nematocysts, the predominant microbasic p-mastigophores and the basotrichous isorhizas. In the present paper we directly measure the discharge of the microbasic p-mastigophores and show that mastigophore-containing cnidocytes are sensitized by representative agonists for these two classes of chemoreceptors. We also show that under certain conditions the number of discharged microbasic p-mastigophores correlates linearly to a major component of the measured adhesive force. This enables us to calculate the contribution to adhesive force made by individual mastigophores.

Introduction

Nearly one hundred years ago, Nagel (1892) suggested that chemicals derived from prey elicit feeding in cnidarians. Recently, two groups of prey-derived chemicals, including N-acetylated sugars and a variety of amino compounds, were identified as being involved in prey capture. In the tentacles of the sea anemone *Aiptasia pal-*

lida, these chemicals act via at least two classes of chemoreceptors to increase the sensitivity of cnidocytes to mechanical stimuli that trigger the discharge of cnidae (Thorington and Hessinger, 1984, 1988a, b). Using mucin-labelled colloidal gold, the receptors for the N-acetylated sugars have been located on the surface of supporting cells adjacent to cnidocytes in the tentacles of the sea anemone, *Haliplanella luciae* (Watson and Hessinger, 1988a, b). As previously discussed (Thorington and Hessinger, 1988a), our working model of the role of cnidocyte-sensitizing chemoreceptors places the N-acetylated sugars as the initial sensitizers that are detected as macromolecular conjugates on the surfaces of prey as mucins, glycoproteins, and chitin. The amino sensitizers, on the other hand, are secondary sensitizers that leak from prey that have been punctured by penetrant nematocysts responding to the conjugated N-acetylated sugars.

The two classes of cnidocyte-sensitizing chemoreceptors were identified in *A. pallida* by measuring cnida-mediated adhesion of tentacles to test probes following appropriate chemical and mechanical stimulation of cnidocytes (Thorington and Hessinger, 1984, 1988a, b). Since adhesive force represents the force required to separate a tentacle from the test probe (Thorington and Hessinger, 1988a), it is an aggregate measure of contributions from all three types of discharging cnidae present in these tentacles. In this paper we show that the majority of the adhesive force is due to the discharge of the microbasic p-mastigophore nematocysts. The cnidocytes housing these nematocysts are chemosensitized to discharge the nematocysts in a dose-dependent manner by the agonists glycine, which represents the amino sensitizing agents, and N-acetylneuraminic acid (NANA),

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which represents N-acetylated sugar agents. These findings validate the underlying assumptions of adhesive force measurements; specifically, that the measured adhesive force is mediated by discharged cnidae, and that the magnitude of adhesive force is proportional to the number of cnidae discharged. From these data we calculate the contribution of individual microbasic p-mastigophores to adhesive force.

Materials and Methods

Maintenance of sea anemones

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, which were cleaned daily. These anemones were maintained on a 12/12 h photoperiod using white fluorescent lights at an intensity of 5.5 klux ($66 \mu\text{Es}^{-1} \text{m}^{-2}$) and ambient temperatures of $24 \pm 1^\circ\text{C}$. Animals were starved for 72 h prior to experiments.

Experimental animals and test solutions

Natural seawater was from the Kerckhoff Marine Laboratory of Caltech at Corona del Mar, California. Experimental conditions and test solutions were essentially as previously reported (Thorington and Hessinger, 1988a). Glycine and N-acetylneuraminic acid (NANA), each representing the amino and the N-acetylated sugar sensitizers, respectively, were tested at specified concentrations in natural, filtered (Whatman type 1) seawater adjusted to pH 7.65 with 1 N HCl or NaOH. The seawater in the finger bowls was replaced with solutions containing specified concentrations of one of these agents. The anemones were allowed to adapt for 10 min before cnidocyte responsiveness was measured.

Assays of cnidocyte responsiveness

Cnidocyte responsiveness to combined chemical and tactile stimulation was determined both by measuring adhesive force and by microscopically counting discharged mastigophores that had adhered to the test probes used to measure adhesive force.

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

weights, and adhesive force measurements were expressed in hybrid units of mg-force (mgf). Each bowl, containing a single sea anemone in the test solution of sensitizer in seawater, was raised by hand until the test probe contacted the tentacle just behind its tip. 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.

Counting discharged mastigophores. After probes had been used to measure adhesive force they were processed as follows so that the mastigophores adhering to them could be counted. The gelatin-coated ends of probes were placed in individual microtiter wells (Microtest 11, Falcon Plastics) containing $40 \mu\text{l}$ of an enzyme and detergent solution (1% Trizyme, Amway Products). The solution was prepared in distilled water, clarified by centrifugation at $2000 \times g$ 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 hydrolyze the gelatin and to release from the probe the mastigophores and other types of cnida, each of which is protease-resistant (Blanquet and Lenhoff, 1966). Probes were then removed and mastigophores remaining in each well enumerated using an inverted light microscope at $200\times$.

Collection and analysis of data

Separate animals were tested at each concentration of sensitizer. Five to ten probes (one per tentacle) were used on each animal to determine both adhesive force and the number of discharged mastigophores. Daily experimental means were calculated from these measurements. Replicate experiments for both glycine and NANA were performed on four different days. 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. Linear regression analysis and determination of maximum response (E_{max}) and of sensitizer concentrations that produce a half-maximum response ($K_{0.5}$) were performed with the aid of a computer-assisted graphics and data-formatting program (Dorgan and Hessinger, 1984).

Results

Glycine as a representative amino sensitizer

The dose-response curves for glycine showing the mean adhesive force (Fig. 1, triangles) and the mean number of discharged mastigophores (Fig. 1, circles) are each biphasic. These dose-response curves coincide somewhat; each has a broad area of sensitization at lower concentrations of glycine, a maximum effect (E_{max}) at about 10^{-6} M glycine, and a broad region of apparent desensitization at still higher concentrations. Three

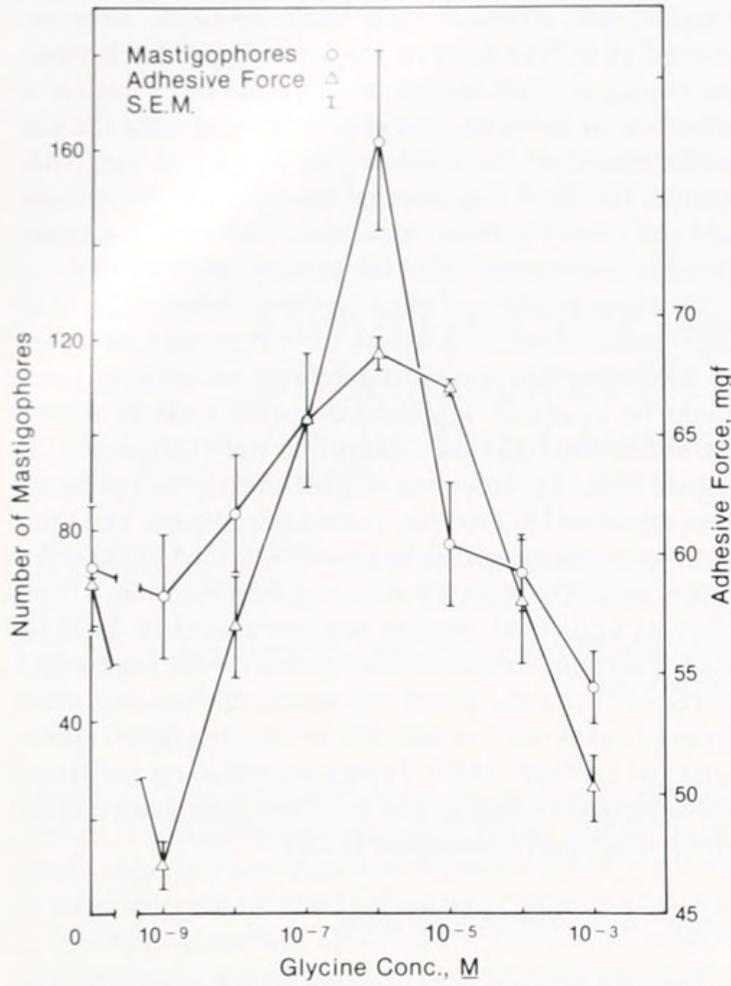


Figure 1. The effects of glycine on adhesive force (mgf) and on the number of discharged mastigophores adhering to test probes. Values for adhesive force (triangles) and for the number of discharged mastigophores (circles) are expressed as means of the daily means of separate measurements with vertical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

differences are apparent. First, the measurement of adhesive force initially decreases at 10^{-9} M glycine before increasing to the maximum effect, while the number of discharged mastigophores shows no such decrease at 10^{-9} M. Second, the magnitude of the maximum increase in adhesive force at E_{\max} versus naive controls is by 15%, whereas the magnitude of the maximum increase in discharged mastigophores is by more than 100% (Fig. 1). Third, the concentrations of glycine which yield the half-maximum effect (*i.e.*, $K_{0.5}$) are somewhat different for adhesive force measurements at 5.0×10^{-8} M than for the number of discharged mastigophores at 1.6×10^{-7} M.

NANA as a representative N-acetylated sugar sensitizer

The effects of different concentrations of NANA on mean adhesive force (Fig. 2, triangles) and on the mean number of discharged mastigophores (Fig. 2, circles) are also biphasic and essentially coincidental. Each dose-re-

sponse curve has regions of sensitization at low concentrations of NANA, E_{\max} values occurring at 10^{-5} M NANA, and regions of apparent desensitization at still higher concentrations. On the other hand, the magnitude of the increase of adhesive force is by 25%, whereas the magnitude of the increase in the number of discharged mastigophores is by nearly 200%. In addition, the concentration to give the half- E_{\max} (*i.e.*, the $K_{0.5}$ value) for adhesive force at 3.2×10^{-7} M is about four times as much as that for the discharged mastigophores at 7.8×10^{-8} M.

Proportionality of nematocysts discharged to adhesive force

The number of discharged mastigophores is directly proportional to the measured adhesive force for sensitizing doses of agonists up to 10^{-6} M glycine and 10^{-5} M NANA (Fig. 3). The calculated line for these data, when extrapolated, intercepts the abscissa to the right of the origin. This indicates that the measured adhesive force consists of at least two components, one that is independent of mastigophores and one that is dependent upon mastigophores. Thus, under these experimentally controlled conditions tentacles exhibit an adhesiveness of

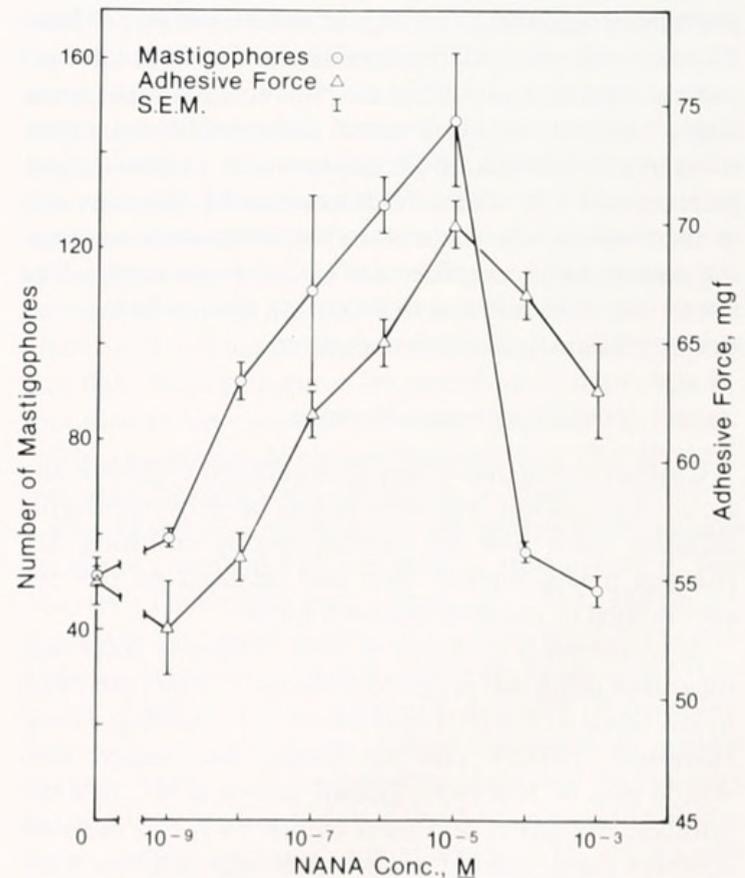


Figure 2. The effects of N-acetylneuraminic acid (NANA) on adhesive force (mgf) and on the number of discharged mastigophores adhering to test probes. Data expressed as in Figure 1.

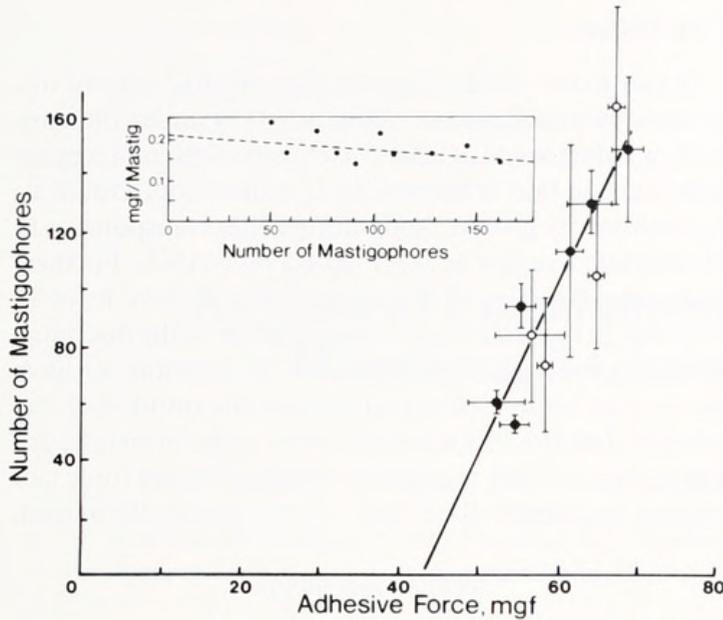


Figure 3. Correlation of discharged mastigophores to measured adhesive force (mgf). Horizontal and vertical bars represent standard errors of the mean (95% confidence limits) for adhesive force and for the number of discharged mastigophores, respectively. Plotted values represent all data measured at sensitizing concentrations of glycine (empty circles) and NANA (filled in circles) from Figures 1 and 2 ($R = 0.92$). Insert: data points obtained by subtracting 43.3 mgf from each mean measurement of adhesive force and dividing these values by the number of discharged mastigophores and then plotting these values as a function of the number of mastigophores discharged. Ordinate-intercept of calculated line (dashed line) is 0.217 ± 0.028 mgf.

approximately 43.3 mgf that is unrelated to the discharge of mastigophores (Fig. 3). This mastigophore-independent component of adhesiveness can be subtracted from each mean measurement of adhesive force. The resulting value can be expressed as the mean, corrected adhesive force per mastigophore. We find that the contribution of each mastigophore to the adhesive force measurement is slightly dependent on the number of mastigophores discharged (Fig. 3, insert). On the other hand, at desensitizing doses of sensitizers the number of discharged mastigophores does not correlate with measured adhesive force (data not shown).

Discussion

Discharge of mastigophores is influenced by two chemoreceptor classes

The adhesion of tentacles to test objects has been used by researchers to detect *in situ* cnida discharge (Williams, 1968; Lubbock, 1979). More recently, using a novel and sensitive technique to quantify adhesion, Thorington and Hessinger (1984, 1988a, b) identified two classes of chemoreceptors that sensitize cnidocytes to discharge cnidae in response to mechanical stimuli.

There are possible limitations, however, associated with using adhesive force to study responsiveness of cnidocytes. One such possible limitation is that measured adhesive force is an aggregate indicator of the discharge of several types of cnidae and, therefore, cannot distinguish between the different types of responding cnidocytes. In the present paper we have shown that a specific type of cnidocyte—those bearing the predominant nematocyst in the tentacles of *A. pallida*, the microbasic p-mastigophore—respond in a biphasic, dose-dependent manner to the chemosensitizers glycine and NANA (Figs. 1, 2, circles). Similar dose-response curves are obtained by measuring adhesive force under identical conditions (Figs. 1, 2, triangles; Thorington and Hessinger, 1988a). Thus, we conclude that the discharge of mastigophores in this anemone is influenced by the two classes of sensitizing chemoreceptors that detect N-acetylated sugars and a variety of amino compounds, including glycine.

Williams (1968), using the sea anemone *Haliplanella luciae*, concluded that the discharge of spirocysts, an adherent and non-penetrating type of cnida, but not that of mastigophores, was sensitized by food extracts. In contrast, using *A. pallida*, we find that the discharge of mastigophores is sensitized by optimum concentrations of both glycine and NANA (Figs. 1, 2, circles), each likely to be constituents of their natural diet. Similar findings for the effects of NANA on the discharge of mastigophores of *H. luciae* have been found by Watson and Hessinger (in press).

In sea anemones the cnidome of the tentacle consists of the adherent spirocysts and the penetrant nematocysts. In acontiate sea anemones such as *A. pallida* and *H. luciae*, the cnidome of the tentacles is made up of spirocysts, microbasic p-mastigophores, and basitrichous isorhizas (Hand, 1955). Of these three types of cnidae the basitrichous isorhizas comprise a comparatively small portion of the total cnidae in the tentacles of *A. pallida* (Giebel, unpub. obs.) and *H. luciae* (Watson and Mariscal, 1983) and are likely to contribute relatively little to the adhesive force measurements.

Dose responses for nematocyst discharge and adhesive force compared

The major difference between the dose-response curves for adhesive force and for the number of discharged mastigophores (Figs. 1, 2) is a proportionally greater increase in the number of discharged mastigophores at E_{max} values than in adhesive force. For example, the maximum increase in number of discharged mastigophores is 100% and 200% for glycine and NANA, respectively, as compared to only 15% and 25% increases in adhesive force, respectively. These differences in max-

imum effects are not surprising since adhesive force is a composite measure of several contributing factors, including cnida-mediated and non-cnida-mediated (*i.e.*, "stickiness") factors, of which the discharged mastigophores is only one.

Contribution of various tentacle factors to adhesive force

The data in this paper show that within the range of sensitizing doses of glycine and NANA, the measurements of adhesive force correlate linearly with the number of discharged mastigophores (Fig. 3). By extrapolating this plot to the abscissa we estimate the adhesive force expected in the absence of discharged mastigophores to be approximately 43 mgf (Fig. 3). Therefore, contributions to adhesive force up to 43 mgf are independent of mastigophores and due to, most probably, a combination of factors, including discharged spirocysts and any inherent "stickiness" of the surface mucus. Recently we obtained measurements for the mucus. The mucus on the tentacle surface contributes approximately 30 mgf to the measure of tentacle adhesive force (Thorington and Hessinger, in prep.) in the absence of cnida discharge.

The difference between 43 mgf (due to surface mucus plus discharged spirocysts) and 30 mgf (due to surface mucus alone) is approximately 13 mgf, possibly accounted for by the sum of all discharged spirocysts. However, this is not to say that the contribution of spirocysts is constant at different concentrations of sensitizer. At sensitizing doses of glycine and NANA, contributory increments in excess of 43 mgf are due to discharged mastigophores. From the slope of the linear correlation between the number of discharged nematocysts and the measured adhesive force we calculate that the contribution of each discharged mastigophore to adhesive force is approximately 0.17 mgf. A comparable value, which is somewhat dependent upon the number of discharged mastigophores, 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 discharged mastigophores (Fig. 3, insert). The slight dependence of the calculated adhesive force per mastigophore upon the number of discharged mastigophores (Fig. 3, insert) is possibly due to a softening effect of discharged mastigophores on the gelatin coating of the probe.

The correlation between number of discharged mastigophores and adhesive force occurs only at stimulatory doses of the tested sensitizing agents. At higher than optimum doses of sensitizer the measurement of adhesive force does not correlate very well with the number of adhering nematocysts, possibly indicating that dramatic changes in other contributions to adhesive force, such as from discharged spirocysts, may also occur.

Conclusions

In summary, we have shown that the discharge of microbasic p-mastigophore nematocysts is under the controlling influence of at least two classes of chemoreceptor systems, one that is responsive to amino compounds as represented by glycine, and another that is responsive to N-acetylated sugars as represented by NANA. Furthermore, the majority of the increase in adhesive force in response to these chemosensitizers is due to the discharge of the microbasic p-mastigophores. In addition, we have shown that under defined conditions the number of discharged nematocysts is proportional to the measured adhesive force. Thus, measurements of adhesive force can be used to quantify the extent of total cnidae discharged.

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