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Stimulus–response coupling in sponge cell aggregation: Evidence for calcium as an intracellular messenger

(Ca channel blockers/calmodulin antagonists/nonsteroidal anti-inflammatory agents/*Microciona prolifera*)

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ABSTRACT **Aggregation of dissociated sponge cells has been proposed as a model for cell–cell recognition mediated by a specific proteoglycan aggregation factor (*Microciona* aggregation factor). To test whether sponge cells undergo stimulus–response coupling in which intracellular Ca is a messenger, aggregation of mechanically dissociated cells was studied. Changes in light transmission through cell suspensions paralleled aggregation as judged by microscopy. In the presence, but not absence, of Ca (>5 mM) partially purified *Microciona* aggregation factor aggregated both living and glutaraldehyde-fixed cells. Evidence for a messenger role of Ca was the following: (*i*) Addition of Ca to Ca-depleted cells induced aggregation that varied with [Ca]. (*ii*) Addition of Ca ionophores (A23187 and ionomycin) caused aggregation that varied with [Ca] and far exceeded that provoked by Ca alone. Glutaraldehyde-fixed cells did not respond to ionophores with or without Ca. (*iii*) Calcium antagonists inhibited aggregation. These included inhibitors of the Ca–calmodulin complex (*N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide hydrochloride and 1-[bis(*p*-chlorophenyl)methyl]-3-[2,4-dichloro-β-(2,4-dichlorobenzyloxyl)phenylethyl]imidazolinium chloride), Ca channel blockers (La, Co, Cd, and verapamil), and three nonsteroidal anti-inflammatory agents (indomethacin, ibuprofen, and piroxicam). Results indicated not only that early events of sponge aggregation can be quantified by continuous recording but that aggregation is not simply due to passive agglutination of inert cells by an extracellular proteoglycan. Rather, sponge cells recognize surface ligands to which they respond by Ca-dependent stimulus–response coupling.**

Since the early studies of Wilson (1) species-specific aggregation of sponge cells has been proposed as a model for cell–cell recognition in embryogenesis. Recently it has been appreciated that aggregation of sponges (e.g., *Microciona prolifera*) is mediated by at least three components: (*i*) a proteoglycan [*Microciona* aggregation factor (MAF)]; (*ii*) a cell surface receptor; and (*iii*) Ca ions (2–4). MAF, a proteoglycan complex of 2×10^7 daltons, is released from cells in Ca,Mg-free seawater (Ca,Mg-FSW) and is irreversibly inactivated by EDTA (2); the surface receptor, "baseplate," is a peripheral membrane protein (5). Dependence of aggregation upon external Ca concentration, $[Ca]_o$, was first documented by Galtsoff (6) and later studied by Humphreys (7), who showed that the effects of Ca were independent of its promotion of cell motility. Moreover, Ca is required for MAF–MAF interactions, whereas the association of MAF with baseplate proceeds in the absence of Ca (4, 8). In each of these studies, it was assumed that Ca affected the surface interactions of aggregating cells. This assumption has been supported by experiments in which Sepharose beads coupled to MAF were aggregated by Ca and in which baseplate-coated beads aggregated in the presence of MAF and Ca; glutaraldehyde-treated cells showed similar specific aggregation patterns (3, 4).

Nevertheless, it appeared possible that the requirement for Ca might also be due to effects of Ca within cells. This possibility was raised by work in our own (9–12) and other (13, 14) laboratories on the role of Ca in the aggregation of human neutrophils.

A role of Ca as an intracellular messenger in stimulus–response coupling has been documented in a variety of cell types—e.g., pancreas (15), salivary gland (16), platelets (17), and nerve cells (18), as well as neutrophils (13, 14). The evidence depends upon the fulfillment of at least five criteria: (*i*) a response to Ca in Ca-depleted cells; (*ii*) a response to Ca ionophores such as A23187 or ionomycin and enhancement of these responses as $[Ca]_o$ is raised; (*iii*) inhibition of the response by Ca antagonists; (*iv*) indirect demonstration of Ca mobilization within cells, which antecedes the response; and (*v*) direct measurements of changes in the cellular Ca concentration, $[Ca]_i$, which antecede the response (see ref. 19). We, and others, have previously shown that the aggregation of neutrophils fulfills each of these criteria (9–11, 13, 14), and in this report we will show that aggregation of sponge cells meets the first three.

MATERIALS AND METHODS

Cells. Specimens of *M. prolifera* were collected by the Marine Biological Laboratory. Sponge fragments (≈1 g) were cut and rinsed 5 min in 30 ml of ice-cold Ca,Mg-FSW (460 mM NaCl/10 mM KCl/7 mM Na_2SO_4/2.5 mM EDTA/10 mM Hepes, pH 7.8). In Ca,Mg-FSW with La, Co, and Cd, Na_2SO_4 was replaced with 10 mM NaCl. Sponge fragments were transferred to 15 ml of Ca,Mg-FSW and cells were dissociated mechanically. Final suspensions contained $\approx 2 \times 10^8$ cells/ml (hemocytometer); 96% of these were not in aggregates (light microscopy). Stock cell suspensions were kept dispersed by maintenance at 4°C.

Aggregation. Into round siliconized glass cuvettes (45 × 4 mm) was placed 0.1 ml of a cell suspension and siliconized metal stirring bar. Aggregation was measured by using a Payton aggregation module (Payton, Buffalo, NY) and an Omniscribe recorder (Houston Instrument, Austin, TX) with stirring at 250 rpm and 22–24°C. Aggregation was recorded as an increase in light transmission (ΔT). Minimal T was set arbitrarily with the cuvette containing 0.1 ml of a suspension. Maximal T was set with 0.05 ml of the suspension and 0.05 ml of Ca,Mg-FSW.

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Abbreviations: MAF, *Microciona* aggregation factor; W-7, *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide hydrochloride; R24571, 1-[bis-(*p*-chlorophenyl)methyl]-3-(2,4-dichloro-β-(2,4-dichlorobenzyloxyl)-phenylethyl]imidazolinium chloride; Ca,Mg-FSW, Ca,Mg-free seawater (with EDTA/Hepes, pH 7.8); EM, electron microscopy.

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Thus, full-scale deflection (20 cm) corresponded to a 2-fold increase in T (100% ΔT). A similar method has been used with human neutrophils (20) and platelets (21) and with *Limulus* amoebocytes (22).

The recordings were quantified by two parameters: (*i*) slope of the curve (% ΔT per min) during rapid aggregation, yielding the *rate* of aggregation, and (*ii*) *extent* of aggregation (% ΔT) from its onset to a constant level (i.e., constant mean of rapid fluctuations).

Electron Microscopy (EM). Cells were fixed in 2% glutaraldehyde in Ca,Mg-FSW immediately after removal from the cuvettes. [Ca] was the same as in the sample (usually 10 mM). Cells were fixed for 5 days and washed three times with Ca,Mg-FSW. Samples were post-fixed with 1% OsO_4 in Ca,Mg-FSW and stained *en bloc* with uranyl acetate. Samples for transmission EM were dehydrated in alcohol and embedded in Spurrs; sections were stained with lead citrate. Samples were examined and photographed with a Zeiss electron microscope 9S. For scanning EM, cells in 70% alcohol were allowed to adhere to polylysine-coated plastic tissue culture wells for 1 hr (23) and then were dehydrated. Samples were dried in a Denton Critical Point Dryer and examined in a JEOL 35U scanning electron microscope at Osborne Laboratories (New York Aquarium). Cells were categorized after Simpson (24) and Wilson and Penney (25).

Cell Viability. This was judged by four criteria: (*i*) trypan blue exclusion [0.5% (wt/vol)]; (*ii*) motility of archeocytes after supravital staining with toluidine blue [0.1% (wt/vol)]; and (*iii*) examination of transmission electron micrographs for the integrity of the plasmalemma and intracellular organelles, including the configuration of the mitochondria. Viability always exceeded 90% of cells in Ca,Mg-FSW with or without Ca and with or without ionophores. Finally, we have confirmed the observation of Humphreys (7) that dead cells (boiled, or in 0.1% Triton X-100) aggregate immediately in the absence of Ca. In all experiments with living cells, aggregation was *not* observed without Ca, and *inhibition* of aggregation by various inhibitors was consistent with the viability of these cells, because the inhibitors did not affect aggregation of dead cells.

Sources of Materials. Materials were obtained as follows: *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide hydrochloride (W-7), Rikaken (Nagoya, Japan); 1-[bis(*p*-chlorophenyl)-methyl]-3-[2,4-dichloro-β-(2,4-dichlorobenzyloxyl)phenylethyl]imidazolinium chloride (R24571), Janssen Pharmaceutica (Beerse, Belgium) and J. E. Brown; verapamil, Knoll Pharmaceuticals (Whippany, NJ); A23187, Eli Lilly (Indianapolis, IN); ionomycin, Squibb Institute (Princeton, NJ); $LaCl_3$, Matheson, Coleman and Bell (Norwood, OH); piroxicam, Pfizer (Groton, CT); ibuprofen, Upjohn (Kalamazoo, MI); and indomethacin, Merck (Rahway, NJ). MAF, antiserum against MAF, and glutaraldehyde-fixed *Microciona* cells were given to us by M. M. Burger. MAF and fixed cells were prepared as described before (2, 5, 26). Its aggregation titer was 1:512 (aggregation at this dilution after 20 min; determined in M. M. Burger's laboratory). An NH_4Cl precipitate of anti-MAF had been purified on a MAF-affinity column.

RESULTS

Aggregation Promoted by Ca. Fig. 1 shows recordings with time of ΔT through suspensions of sponge cells in Ca,Mg-FSW with additions of Ca as indicated. That ΔT, proportional to [Ca], is a measure of aggregation was confirmed by light microscopic examination of material taken immediately from the cuvettes. Before addition of Ca, >96% of cells were dissociated and there were no clumps >100 μm in diameter. After 5 min

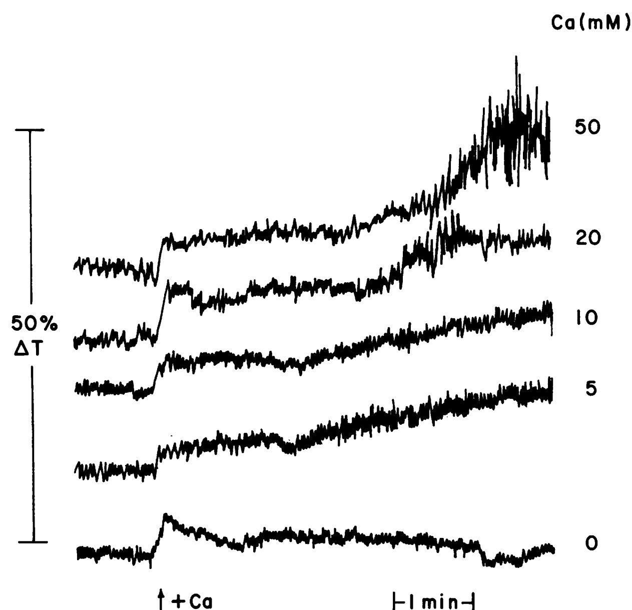

FIG. 1. Effects of varying $[Ca]_o$ on aggregation of Ca-depleted *Microciona* cells: aggregometric tracings of dissociated cells kept for 60 min in Ca,Mg-FSW (including 2.5 mM EDTA). At arrow, where a dilution artifact is noted, 5 μl of $CaCl_2$ was added to 100 μl of cells to a final $[Ca]_o$ as shown. Ordinate represents increase of light transmission (ΔT).

with 10 mM Ca, only 31% of the cells remained dissociated.

Indeed, the records of aggregation and the numbers of aggregates >100 μm observed microscopically (n = 69) showed a direct correlation (data not shown). These results demonstrate that this method can be used to quantify aggregation of *Microciona* cells and confirm earlier demonstrations that Ca added to *Microciona* cells in Ca,Mg-FSW induces aggregation (6, 7).¶

In 24 separate experiments like the one shown in Fig. 1, aggregation at 5 mM Ca exceeded that in Ca,Mg-FSW in both rate and extent ($P < 0.0005$; paired t tests). Aggregation at 10 mM Ca was ≈13% greater than at 5 mM (not statistically significant). $[Ca]_o$ at 20 and 50 mM produced no further increments.

Morphology. Scanning EM of cells in Ca,Mg-FSW shows mainly single cells with relatively few groups of one to three cells (Fig. 2*A*). After treatment with 7.5 μM ionomycin (see below) and 10 mM Ca, large aggregates were formed. In the center of these aggregates were small cells with many extensions and surrounded by larger cells (Fig. 2*B*). Transmission EM revealed a large number of archeocytes and choanocytes (24, 25). Archeocytes have a large nucleus and nucleolus (Fig. 2*D*); choanocytes have a flagellum (Fig. 2*C*).

Enhancement of Aggregation by Ca Ionophores. To test for an intracellular role of Ca in aggregation, cells were incubated briefly with micromolar concentrations of the Ca ionophores A23187 (27) and ionomycin (28) prior to addition of Ca. Fig. 3 shows that A23187 enhanced Ca-induced aggregation and that the rates and extents are proportional to $[Ca]_o$, as with Ca alone (Fig. 1). The same results were obtained with ionomycin at 7 μM. Fig. 4 summarizes the results of many experiments in which the response of cells to 10 mM $[Ca]_o$ is compared with or without the two ionophores.

¶There is uncertainty about Ca activity, a_{Ca}, in the suspensions. EDTA and SO_4 in Ca,Mg-FSW would decrease a_{Ca} of added Ca. However, Ca contributed from fragments of sponge may not all have been removed by the rinse in Ca,Mg-FSW (addition of Ca to 1 mM occasionally induced aggregation).

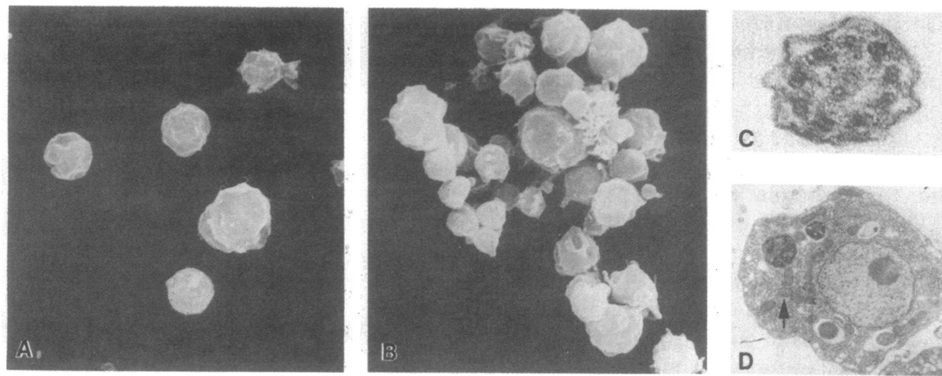

FIG. 2. Scanning (*A* and *B*) and transmission (*C* and *D*) electron micrographs of cells of *Microciona*. Cells were fixed 3 min after addition of Ca,Mg-FSW (*A*) or Ca,Mg-FSW with 7.5 μM ionomycin and 10 mM Ca (*B*). (*A* and *B*, $\times$1,100.) (*C*) Cross section of the flagellum of a choanocyte. ($\times$63,000.) (*D*) Archeocyte with mitochondrion indicated. ($\times$4,000.)

Aggregation Promoted by MAF. MAF has been shown to promote species-specific aggregation (7). Fig. 5 shows that MAF induces aggregation of *Microciona* cells in our experimental system. The uppermost trace, with MAF and Ca, shows greater aggregation than with Ca alone (middle trace). MAF added with no Ca had no effect (lower trace) nor should it because MAF–MAF bonds require Ca and free MAF is unstable with EDTA (2, 4, 8). Jumblatt *et al.* (3) showed that MAF caused aggregation of glutaraldehyde-fixed cells, and it did so in our system as well (5 μl of MAF, rate and extent similar to those with MAF and Ca, live cells, Fig. 5). Neither 5 mM Ca nor 5 μM A23187 and 5 mM Ca induced aggregation of fixed cells.

Effects of Anti-MAF Antiserum. Anti-MAF promoted aggregation of sponge cells in the absence of Ca (Table 1). Preimmune IgG (same rabbit) did not, and anti-MAF enhanced Ca-induced aggregation. If anti-MAF is specific for MAF (this has not yet been shown with rigor), these results are consistent with the presence of MAF on the surface of such cells, despite absence of intact MAF in solution.

Effects of Ca Antagonists. In further tests for an intracellular role of Ca, three classes of Ca antagonists were tested: Ca channel blockers, inhibitors of Ca/calmodulin-dependent processes, and nonsteroidal anti-inflammatory agents. Table 1 shows inhibition by W-7 (specific antagonist of calmodulin; ref. 29) on Ca-induced aggregation. Preincubation for 3 min with 15 μM W-7 before addition of Ca was sufficient to give the full inhibitory effect (not shown), and the inhibition by W-7 was overcome by 50 mM Ca (50 mM Ca after addition of W-7 and 5 mM Ca alone promoted aggregation to the same extent). W-7 has enabled us to distinguish between "agglutination" promoted by extracellular agents and stimulus–response-dependent aggregation: W-7 inhibited aggregation induced by MAF and Ca or Ca alone but did not inhibit anti-MAF-induced aggregation (Table 1). Therefore, Ca and MAF or Ca alone trigger a calmod-

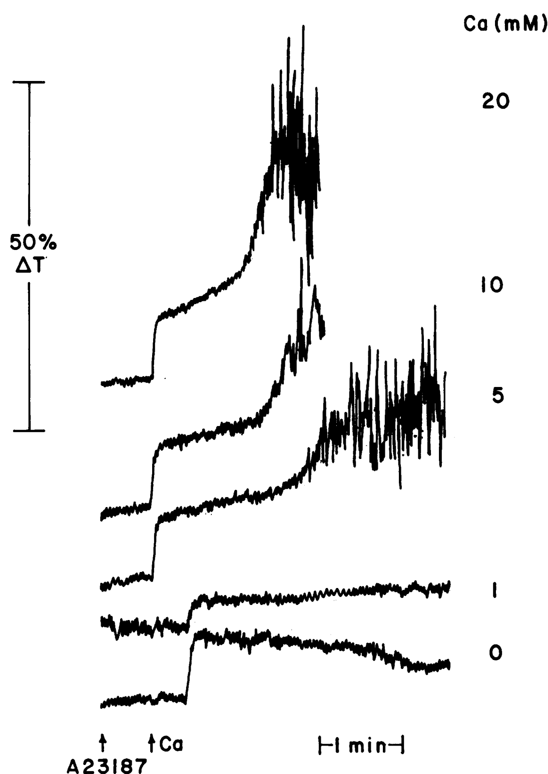

FIG. 3. Effects of varying $[Ca]_o$ on aggregation of dissociated *Microciona* cells induced by the Ca ionophore A23187. Left arrow, 1 μl of A23187 (final concentration = 5 μM) in dimethyl sulfoxide was added to 100 μl cells; right arrow, 5 μl of $CaCl_2$ was added to a final $[Ca]_o$ as shown. Ordinates represent ΔT.

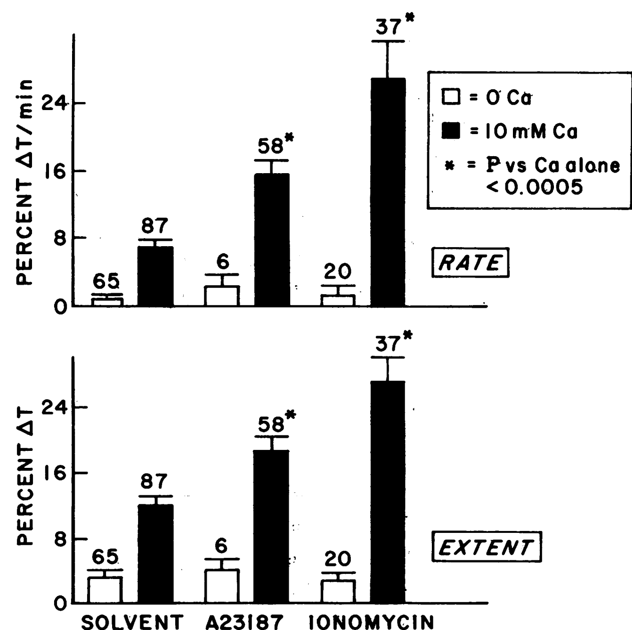

FIG. 4. Effects of Ca and Ca ionophores on aggregation of dissociated *Microciona* cells. Data are expressed as means of rate and extent of aggregation (as shown in Figs. 1 and 2) observed during 5 min after addition of Ca to cells with or without ionophores: 5 μM A23187 or 7.5 μM ionomycin. Numbers of determinations and SEM values are shown above bars.

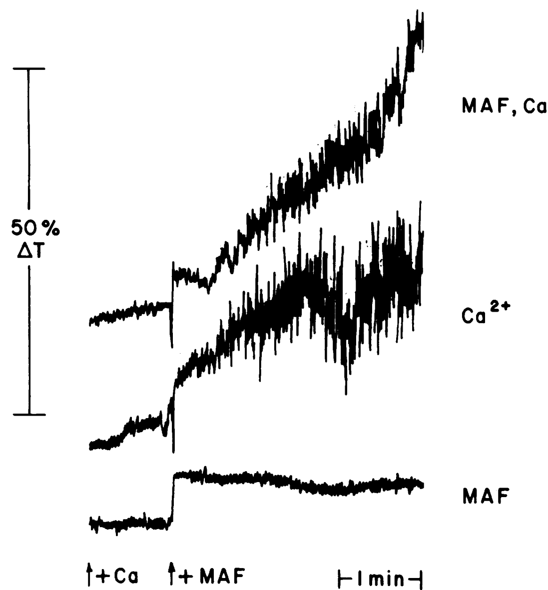

FIG. 5. Effects of MAF with or without Ca on aggregation of dissociated *Microciona* cells. Cells (100 μl) were exposed to $CaCl_2$ (5 μl to give 5 mM final concentration) or to Ca,Mg-FSW (5 μl) at left arrow and to MAF (5 μl, 1:512 titer) or to Ca,Mg-FSW (5 μl) at right arrow.

ulin-dependent process (in addition to their extracellular roles), whereas anti-MAF does not.

R24571, a calmodulin inhibitor that is more active than W-7 (ref. 30), fully inhibited aggregation (5 mM Ca; 5 μM A23187) at 1 μM and retained inhibitory activity at 50 nM. Results with this inhibitor and with the other Ca antagonists are summarized in Table 2. Although complete dose–response curves were prepared for each agent, results are shown only at concentrations that inhibited aggregation by ≥50% ($P < 0.005$, except one instance indicated; paired t tests).

Four Ca channel blockers were tested: verapamil, La^{3+}, Co^{2+}, and Cd^{2+} (refs. 31 and 32); each inhibited aggregation, as shown in Table 2. It has been shown recently that La, Co, and Cd do not inhibit MAF–MAF interactions (33). Indeed, in the absence of Ca, Cd and La could replace Ca in its extracellular role. Therefore, the inhibitory effects of these metals (measured here in the presence of Ca) are not ascribable to an effect on MAF.

Table 1. Effects of MAF, anti-MAF, and the calmodulin inhibitor W-7 on aggregation of dissociated sponge cells

		Aggregation	
Additions to cells*	n	Rate, % ΔT per min	Extent, % ΔT
None	67	0.2 ± 0.4	2.8 ± 1.6
Ca	8	4.8 ± 1.0	10.4 ± 2.0
MAF	6	1.0 ± 1.4	2.2 ± 1.4
+ Ca	7	8.6 ± 2.8†	15.6 ± 2.8†
+ Ca + W-7	5	3.8 ± 2.4	5.4 ± 3.0
Anti-MAF	2	5.8	11.4
+ Ca	2	9.8	16.8
+ Ca + W-7	2	12.6	22.6
Preimmune IgG	2	0	0
+ Ca	2	0	2.0

* Additions to cells were: MAF, 5 μl of partially purified MAF (titer, 1:512); anti-MAF, 5 μl of affinity-purified IgG; preimmune IgG, 5 μl of purified IgG; and 15 μM of W-7. These were added 1 min before addition of Ca to 5 mM. (MAF was added after Ca.) W-7 was added for 3 min before addition of MAF or anti-MAF. Curves from aggregometry recordings were analyzed for rate and extent of aggregation. Data are shown as means ± SEM when $n \geq 5$.

† Greater than Ca alone or MAF + Ca + W-7. $P < 0.025$ for rates and $P < 0.01$ for extents (t tests for two means).

Table 2. Inhibition of *Microciona* aggregation by Ca antagonists

			% inhibition of aggregation induced by A23187 and Ca*	
Inhibitor	Concentration	n	Rate, % ΔT per min	Extent, % ΔT
Ca channel blockers				
Co^{2+}	2.2 mM	3	79.3 ± 5.5	56.3 ± 2.5†
La^{3+}	10.0 mM	5	92.6 ± 1.7	83.6 ± 2.8
Cd^{2+}	2.5 mM	3	94.6 ± 3.0	90.3 ± 10.0
Verapamil	0.1 mM	6	86.0 ± 4.9	66.7 ± 11.0
Calmodulin antagonists				
W-7	15 μM	6	90.2 ± 4.9	79.0 ± 9.5
R24571	1 μM	4	94.3 ± 0.3	91.5 ± 3.0
Nonsteroidal anti-inflammatory agents				
Piroxicam	50 μM	7	61.8 ± 5.4	71.5 ± 3.7
Ibuprofen	50 μM	7	77.0 ± 13.6	80.0 ± 9.7
Indomethacin	50 μM	6	94.7 ± 4.2	89.7 ± 5.4

* Data are expressed as mean (±SEM) for inhibition of rate and extent of aggregation (see Table 1) for means with $n > 3$; for $n = 3$, means are given ± range ÷ 2. La^{3+}, Co^{2+}, and Cd^{2+} (solutions freshly made) were preincubated with cells for 1 min before exposure to 5 μM A23187, followed by 10 mM Ca 1 min after addition of the ionophore. All other inhibitors were preincubated for 5 min before ionophore addition. $P < 0.005$ vs. controls (paired t tests) for all samples, except where noted.

† $P < 0.05$ vs. controls.

Effects on sponge aggregation of three nonsteroidal anti-inflammatory drugs were tested. These agents, which inhibit neutrophil aggregation, presumably by blocking Ca-dependent reactions (ref. 34; H. Korchak, personal communication), inhibited aggregation of sponge cells at 50 μM (Table 2). Again, results are given for only one concentration, but full dose–response curves were obtained.

DISCUSSION

The data illustrate the utility of simple aggregometric techniques, conventionally employed to study platelets (21) or neutrophils (20), for the analysis of early events of sponge aggregation, a process which appears to be an example of stimulus–response coupling. At the very least, the technique can readily be used to establish a more quantitative end point than that employed heretofore: scoring of aggregates visually as 0 to ++++ (4, 5).

Of greater interest is our demonstration that the sponge cell actively participates in its own aggregation: it is not a simple, inert particle that is clumped by a proteoglycan. Three lines of evidence point to this active role of the cells and support the hypothesis that Ca, acting as an intracellular messenger, mediates the process. First, when cells were depleted of Ca—and deprived of soluble MAF—by incubation with EDTA, addition of Ca provoked aggregation. Similar findings have been made for neutrophil activation and have been ascribed to influxes of Ca (9). However, in suspensions of *Microciona*, Ca could simply act by bridging MAF molecules remaining on the surface of cells (5) in the same fashion that it, along with MAF, promoted aggregation of Sepharose beads or glutaraldehyde-fixed cells (refs. 3 and 5 and this work). Evidence for the presence of MAF remaining on the surface of EDTA-treated sponge cells may be provided by our finding that aggregation was provoked by antiserum against MAF, though this conclusion depends on the unproven specificity of the anti-MAF.

The second line of evidence is that the Ca ionophores A23187

and ionomycin induced responses to increases in $[Ca]_o$ far greater than those to Ca alone. Because extracellular MAF–MAF interactions cannot depend upon the ionophore-mediated increase of $[Ca]_i$ enhanced aggregation by ionophores is strong evidence that $[Ca]_i$ plays a role in sponge aggregation. This hypothesis is supported by failure of ionophores (with or without Ca) to promote the aggregation of fixed cells.

Finally, we have found that three classes of Ca antagonists block aggregation induced by ionophores or MAF. These include (*i*) Ca channel blockers: verapamil, La, Cd, and Co; (*ii*) two inhibitors of the Ca–calmodulin complex: W-7 and R24571; and (*iii*) three nonsteroidal anti-inflammatory agents. Each of the three nonsteroidals—at ID_{50} values of $\approx$50 μM—inhibit neutrophil aggregation provoked by a chemoattractant (35) and mobilization of membrane-associated Ca, as reflected by decrements in the fluorescence of chlorotetracycline-loaded cells (34). In addition, indomethacin inhibits ^{45}Ca uptake by stimulated neutrophils (H. Korchak, personal communication).

Thus, ionophores that promote Ca influx *enhance* sponge aggregation, whereas agents that block the entry of Ca, or which modulate its action within cells, *inhibit* aggregation. In consequence, the results suggest that this process meets three of the five criteria proposed in the Introduction for the dependence upon $[Ca]_i$ of stimulus–response coupling and should lead to further tests of the remaining criteria.

Therefore, Ca seems to play an extracellular role in sponge aggregation and an intracellular role as well (apparently mediated via calmodulin). In this context, the concept of agglutination may be useful—a process mediated by a defined "agglutinin" in the sense of Lillie (36), first employed with respect to sponge aggregation by Galtsoff (37). *Microciona* aggregation may be described as the sum of two processes. The first, agglutination, is exemplified by MAF acting to promote aggregation of fixed cells or baseplate-coated Sepharose beads. The second, stimulus–response coupling or "triggering," depends upon MAF acting as a ligand at its receptor, baseplate, and triggering a calmodulin-dependent event within the cell, perhaps by permitting Ca entry. Ionophores, as in the neutrophil (10–12), bypass ligand–receptor interactions and trigger the cells directly. These two processes can also be distinguished in the case of sponge cells aggregated by anti-MAF, which is not inhibited by W-7, thereby reflecting agglutination without triggering. In contrast, aggregation by MAF—acting as a ligand as well as an agglutinin—is blocked by W-7. Agglutination is mediated by extracellular Ca, whereas triggering is mediated by Ca as an intracellular messenger. The ultimate response to triggering is of course aggregation. The proximal response is unidentified but is probably secretion of MAF.

Elucidation of the intracellular events transduced by $[Ca]_i$ should permit analyses not only of the aggregation of sponges but also of the later adumbrations of this process in cells of higher organisms. But even this suggestion was anticipated by Galtsoff (6) who wrote in 1925: "The coalescence of separated sponge cells has some resemblance to the agglutination of extravasated blood cells. Apparently in both cases we are dealing with the interaction of motile cells taken from the normal condition inside the organism and put into a new environment."

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1. Wilson, H. V. (1907) *J. Exp. Zool.* **5**, 245–258.
2. Cauldwell, C. B., Henkart, P. & Humphreys, T. (1973) *Biochemistry* **12**, 3051–3055.
3. Jumblatt, J., Weinbaum, G., Turner, T., Ballmer, K. & Burger, M. M. (1977) in *Surface Membrane Receptors*, eds. Bradshaw, K., Frazier, U., Merrel, R., Gottlieb, D. & Hogue-Angeletti, R. (Plenum, New York), pp. 73–86.
4. Misevic, G. N., Jumblatt, J. E. & Burger, M. M. (1982) *J. Biol. Chem.* **257**, 6931–6936.
5. Jumblatt, J. E., Schlup, V. & Burger, M. M. (1980) *Biochemistry* **19**, 1038–1042.
6. Galtsoff, P. S. (1925) *J. Exp. Zool.* **42**, 183–221.
7. Humphreys, T. (1963) *Dev. Biol.* **8**, 27–47.
8. Henkart, P., Humphreys, S. & Humphreys, T. (1973) *Biochemistry* **12**, 3045–3050.
9. Goldstein, I. M., Horn, J. K., Kaplan, H. B. & Weissmann, G. (1974) *Biochem. Biophys. Res. Commun.* **60**, 807–812.
10. Smolen, J. E., Korchak, M. M. & Weissmann, G. (1981) *Biochim. Biophys. Acta* **677**, 512–520.
11. Smolen, J. E. & Weissmann, G. (1982) *Biochim. Biophys. Acta* **720**, 172–180.
12. Hoffstein, S. & Weissmann, G. (1978) *J. Cell Biol.* **73**, 242–251.
13. Naccache, P. H., Showell, H. J., Becker, E. L. & Sha'afi, R. I. (1979) *J. Cell Biol.* **83**, 179–186.
14. Naccache, P. H., Molski, T. F. P., Alobaidi, T., Becker, E. L., Showell, H. J. & Sha'afi, R. I. (1980) *Biochem. Biophys. Res. Commun.* **97**, 62–68.
15. Chandler, D. E. & Williams, J. A. (1978) *J. Cell Biol.* **76**, 386–394.
16. Rasmussen, H. (1970) *Science* **170**, 404–412.
17. Feinstein, B. B. (1980) *Biochem. Biophys. Res. Commun.* **93**, 593–600.
18. Hallet, M., Schneider, A. S. & Carbone, E. (1972) *J. Membr. Biol.* **87**, 23–32.
19. Weissmann, G., Smolen, J. E., Korchak, H. M. & Hoffstein, S. (1981) in *Cellular Interactions*, eds. Dingle, J. T. & Gordon, J. (Elsevier/North-Holland, Amsterdam), pp. 15–31.
20. Kaplan, H., Edelson, H., Friedman, R. & Weissmann, G. (1982) *Biochim. Biophys. Acta* **721**, 55–63.
21. Born, G. V. R. & Cross, M. J. (1963) *J. Physiol. (London)* **168**, 178–195.
22. Kenney, D. M., Belamarich, F. A. & Shepro, D. (1972) *Biol. Bull.* **143**, 548–567.
23. Hoffstein, S. T., Friedman, R. S. & Weissmann, G. (1982) *J. Cell Biol.* **95**, 234–241.
24. Simpson, T. L. (1963) *J. Exp. Zool.* **154**, 135–151.
25. Wilson, H. V. & Penney, J. T. (1930) *J. Exp. Zool.* **56**, 73–147.
26. Humphreys, S., Humphreys, T. & Sano, Y. (1977) *J. Supramol. Struct.* **7**, 339–351.
27. Pressman, B. C. (1976) *Annu. Rev. Biochem.* **6**, 501–530.
28. Liu, W. C., Slusarch, D. S., Astle, G., Trejo, W. H., Brown, W. E. & Meyers, E. (1979) *J. Antibiot.* **31**, 815–819.
29. Kobayashi, R., Tawata, M. & Hidaka, H. (1979) *Biochem. Biophys. Res. Commun.* **88**, 1037–1045.
30. Gietzen, K., Wuthrich, A. & Bader, H. (1981) *Biochem. Biophys. Res. Commun.* **101**, 418–425.
31. Baker, P. F., Meves, H. & Ridgway, E. B. (1973) *J. Physiol. (London)* **231**, 511–526.
32. Kostyuk, P. G. & Krishtal, O. A. (1977) *J. Physiol. (London)* **231**, 511–526.
33. Rice, D. J. & Humphreys, T. (1983) *J. Biol. Chem.* **258**, 6394–6399.
34. Edelson, H. S., Kaplan, H. B., Korchak, H. M., Smolen, J. E. & Weissmann, G. (1982) *Biochem. Biophys. Res. Commun.* **104**, 247–253.
35. Edelson, H. S., Kaplan, H. B. & Korchak, H. M. (1982) *Clin. Res.* **30**, 469 (abstr.).
36. Lillie, F. R. (1919) *Problems of Fertilization* (Univ. of Chicago Press, Chicago), pp. 1–278.
37. Galtsoff, P. S. (1923) *Biol. Bull.* **45**, 153–161.