CALCIUM CONTROL OF CILIARY ARREST IN MUSSEL GILL CELLS

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ABSTRACT

After several hours in 20 mM sodium phosphate and 40 mM KCl (pH 7.4) or similar simple solutions, ciliated cells exfoliate en masse from stripped gill epithelium of freshwater mussels, e.g., *Elliptio complanatus*. Three types of ciliated cells—lateral (L), laterofrontal (LF), and frontal (F)—can be distinguished and counted separately in the suspensions. About one-half of the cells of each type remain motile. Motility is unaffected by addition of 10^{-5} M A23187 or 10^{-2} M Ca^{+2} added separately, but when ionophore and Ca^{+2} are added together, ciliary beat is largely arrested.

Treatment of the cells with Triton X-100 (Rohm & Haas Co., Philadelphia, Pa.) results in a total loss of motility as the ciliary membrane becomes disrupted. Such models can be reactivated by addition of ATP and Mg^{+2}. All ciliated cell types are reactivated to about the same extent. At least 80% of the activity of the untreated preparation returns. Ca^{+2}-EGTA buffers added to the reactivating solutions permit titration of free Ca^{+2} concentration vs. percent motility. Activity is unchanged for all cell types at $Ca^{+2} < 10^{-7}$ M; at 10^{-6} Ca^{+2}, L cilia are arrested differentially, whereas at $Ca^{+2} > 10^{-4}$ M most cilia of all cell types are arrested. We conclude: (*a*) that increasing cytoplasmic Ca^{+2} is directly responsible for ciliary arrest, (*b*) that the readily reversible physiological arrest response of the L cilia in the intact gill is caused by a rise in free Ca^{+2} in narrow limits from ca. 5×10^{-7} M to ca. 8×10^{-7} M, and (*c*) that the site which is sensitive to Ca^{+2} is part of the ciliary axoneme or the basal apparatus.

KEY WORDS cilia · calcium · A23187 · cell motility · lamellibranch gill epithelium

Ciliary arrest or similar behavioral responses have been observed for many metazoan epithelia (7), but arrest has been characterized best for the lateral cilia of lamellibranch gill cells, e.g., *Mytilus* (25, 26) and *Elliptio* (19, 20). The response may be produced by chemical, mechanical, or electrical (9) stimulation, or by laser irradiation (8, 21). In the latter case, a transient arrest spreads from cell to cell, and undamaged cilia eventually recover normal beat.

Murakami and Takahashi (10) have recently reported that the arrest response in *Mytilus* gill is accompanied by membrane depolarization. This, taken together with the results of Satir (19) and Murakami and Takahashi (11), suggests that the basic mechanism producing arrest is similar to that producing ciliary reversal in *Paramecium*. As is

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well known for *Paramecium*, reversal is coupled to a voltage-dependent depolarization of the cell membrane and a Ca^{+2} flux across the membrane (4, 12).

The important role of intracellular calcium ion concentration controlling the behavior of cilia and flagella has been well established not only for *Paramecium* (13, 14), but also in *Crithidia* (5), sea urchin sperm (3), and *Chlamydomonas* (6). These studies have shown that the behavior of membraneless ciliary axonemes reactivated with ATP and Mg^{+2} changes upon addition of appropriate amounts of free Ca^{+2} to the solution. However, this has not been thoroughly demonstrated for the responses of metazoan epithelia such as ciliary arrest.

Satir (19) used a different approach to this problem. He showed that the lateral cilia of *Elliptio* could be arrested in the presence of the divalent cationic ionophore A23187 and high concentrations of external Ca^{+2}, but that neither ionophore nor external Ca^{+2} alone was capable of producing arrest. Interestingly, under these conditions, other ciliated cells of the gill still beat, as is also usually the case when arrest is produced by physiological stimulation. These experiments were performed on excised gill filaments, and in such cases it is not possible to eliminate entirely indirect effects on the underlying nonciliated tissue elements. More complex but essentially similar effects of Ca^{+2} ionophores on lateral cilia of *Mytilus* have been obtained by Murakami and Takahashi (11).

In this paper, we address this question in a new way. We demonstrate the possibility of disintegrating the epithelium while retaining the motility of the cilia. We will show (*a*) that these isolated ciliated gill cells show the same ionophore-Ca^{+2}-induced arrest response as the intact gill filament, (*b*) that this response is completely explained by the assumption that these agents cause an increase in cytoplasmic Ca^{+2}-concentration, (*c*) that the amount of Ca^{+2} required to produce arrest can be titrated, such that the differential sensitivity of the lateral cilia can be explained, and (*d*) that Ca^{+2} acts directly on the ciliary axoneme.

A preliminary report of these findings was presented at the Meeting of the Biophysical Society in New Orleans (29). While this work was in progress, Tsuchiya (27, 28) studying single reactivated *Mytilus* gill filaments reported the arrest of Triton-extracted lateral cilia in the presence of high Ca^{+2}.

MATERIALS AND METHODS

Preparation of Isolated Gill Cells

Suspensions of cells from the gill of *Elliptio complanatus* or related genera were prepared by placing pieces of stripped gill tissue (18) into buffer solutions, referred to as isolation medium A or B. Isolation medium A contained 20 mM sodium phosphate buffer and 40 mM potassium chloride. Isolation medium B consisted of 20 mM sodium chloride, 40 mM potassium chloride, and 40 mM *N*-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid (HEPES). The pH of both isolation media was 7.4. The intact gill was kept for 3–4 h in the media at room temperature. After 2-4 h, cells exfoliate in large numbers from the epithelium. Three types of ciliated cells are readily distinguished and can be counted separately when appropriate: lateral (L), laterofrontal (LF), and frontal (F) cells (see Figs. 1 and 2) (17). The rarer abfrontal cells bearing cilia are not distinguished from F cells in this study.

Cell suspensions from isolation medium A were used for the preparation of gill cell models and for the determination of the critical calcium concentration for ciliary arrest, whereas cells isolated in medium B were used for the studies with ionophore. Cells were examined in a Zeiss photomicroscope under phase contrast. Aliquots of cell suspensions were scored for motility with cells counted individually in a modified differential count. A multiciliated cell was considered motile when >50% of its cilia were estimated to be beating.

Preparation of Ionophore

Ionophore A23187 was obtained from Eli Lilly & Co., Indianapolis, Ind. (courtesy of R. Hamill) and was prepared as 10^{-3} M stock solution in 0.25% dimethyl formamide and 0.75% ethanol. Cells from isolation medium B were used, and ionophore diluted in isolation medium B was added to a final concentration of 10^{-5} or 10^{-6} M. Cells were then scored for motility in the light microscope.

Preparation of Cell Models

The techniques used for preparing detergent-extracted models are similar to those described by other investigators (13, 24). Cell suspensions in isolation medium A were filtered twice through cheesecloth and centrifuged for 5 min at 600 *g* in a clinical centrifuge. The cell pellet was resuspended for 5–10 min in extraction solution; this solution consisted of 0.04% Triton X-100 (Rohm & Haas Co., Philadelphia, Pa.), 100 mM potassium chloride, 2 mM EDTA, 30 mM HEPES, pH 7.0. The cells were washed twice in wash solution containing 50 mM KCl and 30 mM HEPES at pH 7.0. Aliquots from this solution were then observed under the light microscope, and cells were scored for motility. Reactivation solution was added, and the cells were again scored for motility. The reactivation solution was com-

posed of 100 mM KCl, 2 mM ethylene glycol-bis(β-aminoethyl ether)*N*,*N*′-tetraacetic acid (EGTA), 8 mM $MgCl_2$, 4 mM ATP, and 30 mM HEPES at pH 7.0. ATP was obtained from Sigma Chemical Co., (St. Louis, Mo.) as the disodium salt.

To study the influence of calcium on the reactivation of cell models, cells were prepared as described in the previous paragraph. After the cells were resuspended in wash solution, aliquots were taken and reactivation solution containing calcium in the range of 10^{-8} to 10^{-2} M was added and cells were scored for motility in the light microscope. Motility was also tested when cells were already reactivated and calcium at the appropriate concentration was added later. The concentration of free calcium was controlled by EDTA and EGTA and estimated after the methods of Portzehl et al. (15). Additionally, reactivation solutions were also measured directly against known EGTA-Ca^{+2} buffers in terms of murexide difference spectra (23). We thank William Reed for help with these calculations and measurements. We use the notation pCa to stand for $-\log [Ca^{+2}]$. Both reactivation and Ca^{+2}-induced arrest were also observed on individual cells which were placed under the microscope in a control solution (e.g., containing no ATP, containing 10^{-8} M Ca^{+2}, etc.). The test solution was then drawn under the slide by capillary action. The final concentration of substances in the test solution at the site of action cannot be controlled in this way, but critical information on individual ciliary response is obtained.

RESULTS

Cell Suspension of Isolated Cells

Fig. 1 shows isolated cells from *Elliptio* gill epithelium after 3 h in isolation medium. In the intact gill tissue, there are three main ciliated cell types, distinguished by the arrangement and the length of their cilia (17): L cells contain about 200, 14-μm long individual cilia; F cells are also multi-ciliated but they are somewhat smaller than L cells, and F cilia are only about 1/3 as long. The LF cilia are grouped into a characteristic sail-shaped cirrus. These three cell types can also be distinguished in the cell suspension (Fig. 2) so that the origin of a ciliated cell can be determined. A given aliquot of isolated ciliated cells is composed of ca. 20% L cells, 40% LF cells, and 30% F cells (Table I). (Nonciliated cells or cells that have lost their cilia are not included in this total). Electron microscopy of the isolated cells (Fig. 3) showed that these cells contain the usual complement of organelles with relatively normal morphology and that the cell and ciliary membranes are usually intact.

Observation of cell suspensions showed that many of the isolated ciliated cells remain motile for several hours at room temperataure. As far as we have determined, beat form in the L and F cilia is reasonably normal. The cilia in the cirrus are coordinated to produce a twitch or general flexure of the organelle. The cells do not swim, but rather rock more or less in place, making counting possible. Such cell suspensions have been scored for motility (Table I). In isolation medium A, 51% of the overall ciliated cells were motile, and in medium B, 55%. The motility of the three cell types was determined individually (Table I). In general, L cell motility appears somewhat more labile than F or LF cell motility. Motility of the LF cells is apparently decreased in medium A versus B, but motility in the other cell types is unaffected. Motile vs. nonmotile cells are not readily distinguishable morphologically in either light or electron microscopy.

Effect of External Ca^{+2} and Ionophore on Isolated Gill Cells

We have repeated the work of Satir (19) using the isolated cell suspension to show that the effect of Ca^{+2} and ionophore in producing arrest is on the gill cells themselves. The L cilia arrest response observed on excised gill epithelium can be reproduced with the isolated cells, but, in addition, we can successfully arrest LF and F cells also.

Ionophore A23187 at a final concentration of 10^{-5} M added to cells suspended in isolation medium B—i.e., in the presence of 20 mM Na^+ and 40 mM K^+—did not alter overall motility. Likewise, no significant effect on motility could be detected when Ca^{+2} at a concentration of 12.5 mM was added to the medium. Isolated cells were also tested in the solvents for ionophore and no effect was observed. However, when ionophore and Ca^{+2} were added together, a drastic reduction of percent motility was observed (Table II). All cell types were affected, although L cells appear perhaps slightly more sensitive. Arrest is incomplete in that about 10% of the L cells and 15% of LF and F cells show continued motility. There is variability between individual mussels (Table III) but usually the effects are qualitatively the same.

Extraction and Reactivation

The detergent treatment of the ciliated gill cells resulted in a total loss of motility. Cilia stopped

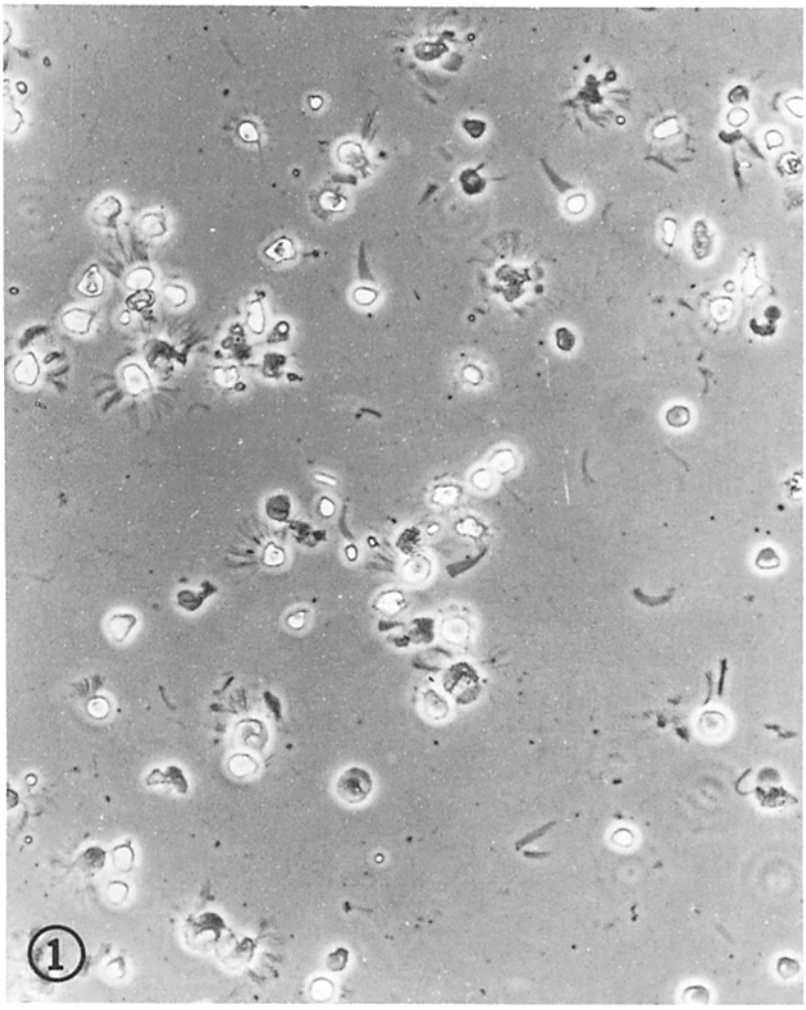

FIGURE 1 Overview of field of cells which have exfoliated from gill tissue after 3 h in isolation medium. Many individual cells are identifiable; about half of these are motile. × 300.

beating and usually straightened or stiffened (Fig. 4*a*) within 3–5 min after the extraction solution was added. This treatment was monitored in the light microscope. We found that some of the cells retain their normal appearance, whereas others lose the bulk of their cell bodies, leaving only the ciliated cortex as an identifiable array (Fig. 2). We call these latter structures "eyelashes." The cell origin of the eyelashes can still be determined from cilium length and appearance. In further experiments, the eyelashes behave the same way as the cell models that have retained their bodies and, therefore, are not separated in our counts. After treatment with detergent, the membranes of cells and cilia are greatly disrupted, but never completely absent, even though single axonemal cross sections may be membrane-free. The ultrastructure of the axoneme is well preserved (Fig. 3). Dynein arms and radial spokes are visible. Some of the arms extend between doublets. Despite the light microscope appearance of ciliary rigor, the rigor position of the arms is not readily maintained after these procedures.

To samples of the extracted cell models under the light microscope, reactivation solution was added. When the reactivation solution reached the cells, the cilia started moving immediately (Fig. 4*b*). The extended rigor form of the cilium gives way to a variety of normal beat stages, presumably as normal levels of ATP and Mg^{+2} are

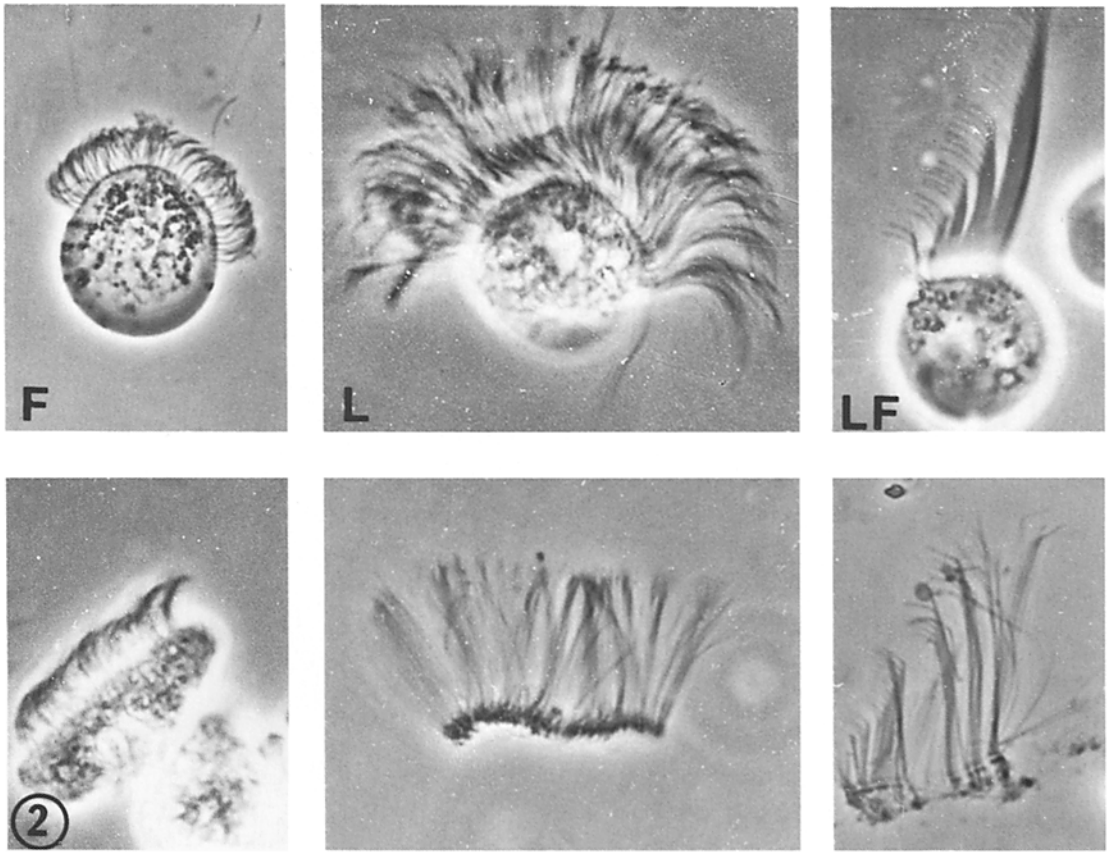

FIGURE 2 Ciliated cell types (above) and corresponding eyelashes (below) are readily distinguished: *F*: frontal cells. These bear numerous 5 μm long individual cilia. *L*: lateral cells. These bear 14 μm long individual cilia. *LF*: laterofrontal cells. These bear two sail-shaped cirri consisting of fused cilia of a sequence of differing lengths. Above, × 1,300; below, × 1,500.

TABLE I

Motility of Isolated Ciliated Cells of Elliptio Gill

	Isolation medium A			Isolation medium B		
Cell type	Motile	Nonmotile	% Motility	Motile	Nonmotile	% Motility
L	253	219	53	139	164	46
LF	247	376	40	229	149	60
F	297	180	62	125	81	61
Total Ciliated Cells	797	775	51	493	394	55

See Materials and Methods for composition of isolation media A and B. Within a single microscope field, L, LF, and F cells are scored separately as motile or nonmotile, and % motility is calculated. The total number of cells counted represents 18 experiments in isolation medium A and 8 in medium B.

restored to the axoneme. If either ATP or MgCl is omitted from the reactivation solution, reactivation does not occur. In all, about 40–50% of the cells in the preparation are reactivatable. Table IV shows that (*a*) all ciliated cell types are reactivatable to approximately the same extent and that (*b*) at least 80% of the activity of the untreated preparation returns. We are unable to

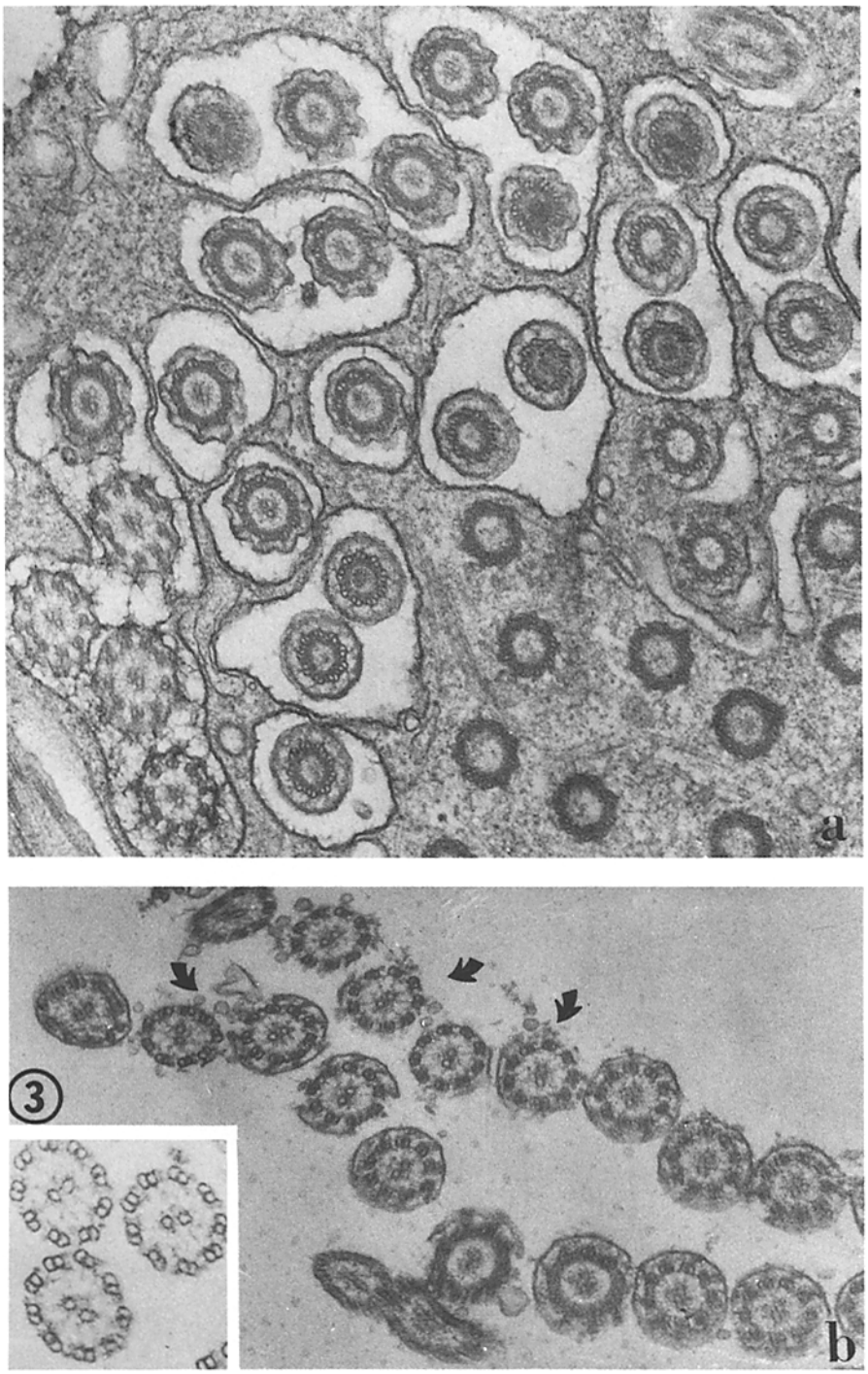

FIGURE 3 (*a*) Isolated L cell cortex showing numerous basal bodies. General appearance of the cytoplasm is normal; cell and ciliary membranes are intact. × 50,000. (*b*) Isolated LF cell after triton treatment. Arrows indicate disruption of the cell membrane. × 60,000. (*Inset*) Isolated L cell cilia. At this point on the axoneme, membrane has been dissolved away completely. Note preservation of normal axonemal appearance, including evident arms and spokes. The arms are sometimes, but not consistently, attached to the adjacent subfiber B. × 74,000.

TABLE II

Effect of Ionophore A23187 and Ca^{+2} on Isolated Gill Cells in Isolation Medium B

Cell type	% Motility			
	Medium alone	+A23187 only	+$CaCl_2$ only	+(A23187 + $CaCl_2$)
L	42 ± 7.0	46 ± 13.0	53 ± 10.1	9 ± 6.1
LF	56 ± 7.2	59 ± 13.2	60 ± 11.9	15 ± 8.9
F	58 ± 11.6	53 ± 8.2	62 ± 9.8	17 ± 11.2

Each value ± SD represents eight experiments; A23187 is added at 10^{-5} M; $CaCl_2$ at 10^{-2} M.

TABLE III

Comparative Effects of Ionophore A23187 and Ca^{+2} on Isolated Ciliated Cells of Two Gills from Different Mussels

	% Motility											
	Medium alone			+A23187 only			+$CaCl_2$ only			+(A23187 + $CaCl_2$)		
Cell type	L	LF	F	L	LF	F	L	LF	F	L	LF	F
Mussel 1	32	52	56	38	48	58	46	65	57	4	9	8
Mussel 2	42	60	56	42	42	45	51	67	60	10	17	20

say whether only cells that were active when isolated are reactivatable, but we assume that it is usually such cells that we see.

Ca^{+2} Induced Arrest of Model Cilia

Fig. 4*c* and *d* show a reactivated L cell respectively before and after addition of 12.5 mM $CaCl_2$ to the reactivation medium. Addition of Ca^{+2} at concentrations approximately 10^{-2} M reverses reactivation and causes the beating cilia of all cell types to stop. The L cilia in Fig. 4*d* again generally assume a straight rigor-like configuration. We have investigated the effect on motility of addition of various concentrations of Ca^{+2} to the reactivation solution. In Table V, we compare the effect of selected concentrations of free Ca^{+2}, added as Ca-EGTA buffers (see Materials and Methods), on motility of the ciliated cell models and eyelashes from two different gills. In reactivation solution alone (i.e., containing 4 mM ATP and 8 mM Mg^{+2}), the two gills reactivate in the usual manner; however, in these cases, reactivation of L cilia was somewhat difficult to obtain. Activity is essentially unchanged for all cell types in the lowest level of Ca^{+2} added (pCa >7), whereas at higher levels (pCa <6) L cilia are arrested differentially. The qualitative results for both gills are similar. At pCa <6, moderate effects are usually seen on LF and F cells, but for single experiments these are obscured by the variability in the data. In control experiments, where Na^+ in place of Ca^{+2} is added together with EGTA, reactivation is not inhibited.

More complete information regarding Ca^{+2}-induced inhibition of reactivation is contained in the titration curves of Fig. 5. Here, it is apparent that L cilia are differentially arrested such that about three-fourths of reactivatable L cells are arrested when free Ca^{+2} changes within narrow limits from ca. 5×10^{-7} M to 8×10^{-7} M. In contrast, for LF and F cilia, this change has a minimal effect and three-fourths of the reactivatable LF and F cells are arrested only at a concentration of free Ca^{+2} at least two orders of magnitude higher (pCa <4). Thus, a change in cellular free Ca^{+2} from 10^{-7} to 10^{-6} M might be expected to arrest the L cilia (and metachronism) without appreciably stopping LF or F ciliary beat. Because "eyelashes" are also arrested, the effect of this change in Ca^{+2} is directly on the ciliary complex itself.

DISCUSSION

Ciliary arrest is an important response of mussel gill epithelium. It has been most thoroughly studied in L cells, where its physiological significance seems to be that water currents to the organism can be shut down, either momentarily or for longer periods, under unfavorable environmental conditions. A transient arrest has also been produced experimentally in LF cells, for example, by laser microsurgery (8). Earlier workers have considered such arrests to be caused by an in-

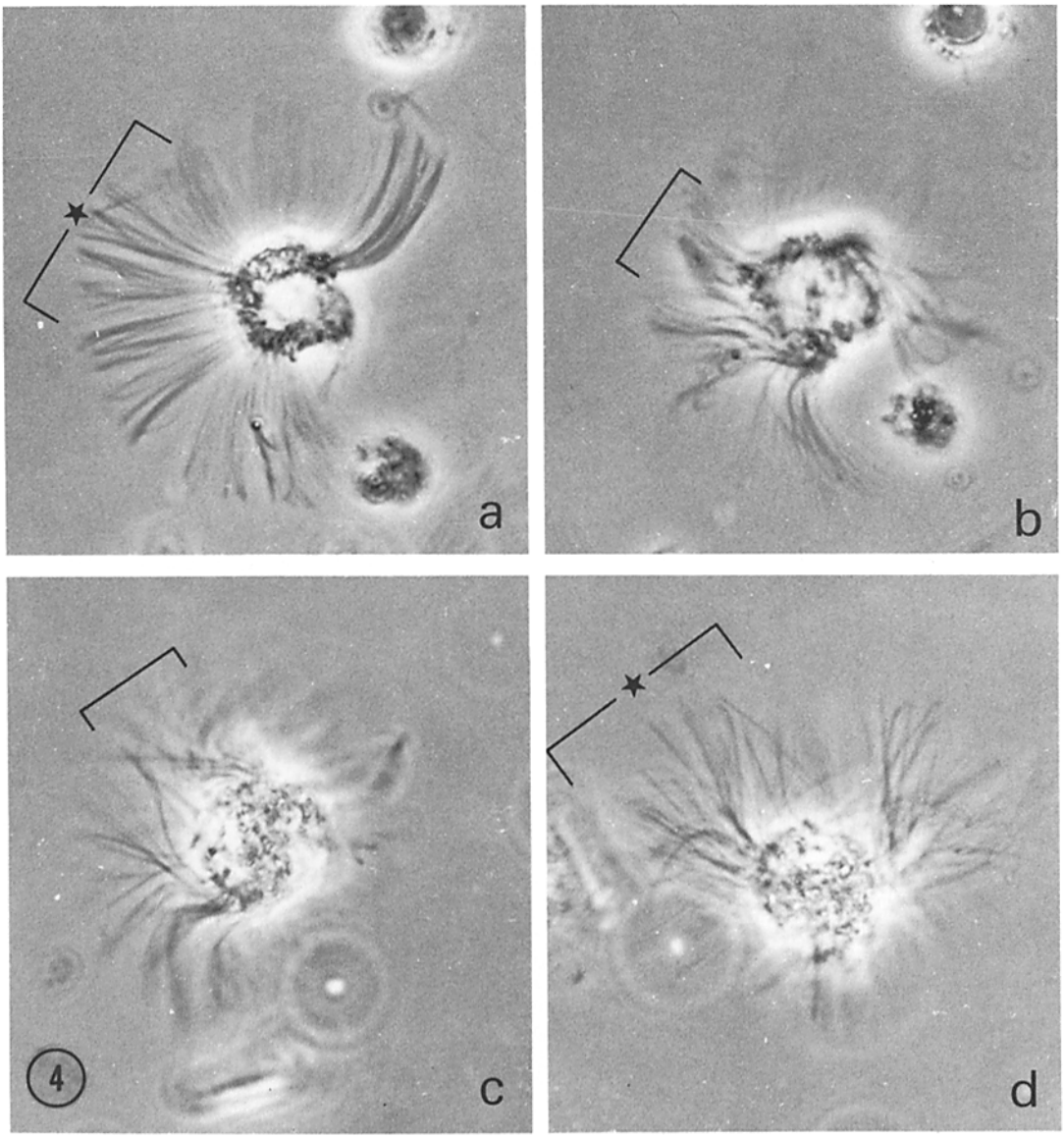

FIGURE 4 (*a* and *b*) Reactivation experiment, before (*a*) and after (*b*) addition of ATP. Note straight (rigorlike?) appearance of L cilia in Fig. 4*a* before ATP has reached the membraneless axonemes (bracketed star). The cilia in the same region in Fig. 4*b* are largely out of the plane of focus because they are beating. × 1500. (*c* and *d*) Ca^{+2}-induced arrest of model cilia, before (*c*) and after (*d*) addition of ca. 10^{-2} M Ca^{+2}. In Fig. 4*c*, the cell is reactivated; in Fig. 4*d*, the cilia in the same region are once again generally straight (bracketed star) and they have stopped beating. × 1500.

crease in free cytoplasmic Ca^{+2} concentration, but, for the most part, evidence for this conclusion has been indirect. Some of the most suggestive evidence for this view comes from the work of Satir (20), who showed that, in gill filaments of the freshwater mussel *Elliptio*, reversible arrest of L cilia could be produced by perfusing the gill with 10^{-5} M A23187 and 12.5 mM Ca^{+2}. One difficulty with this study, which utilizes the entire excised gill filament, and with the similar study of Murakami and Takahashi (11) on *Mytilus*, is that the tissue is heterogeneous and components other

than the ciliated epithelial cells themselves (i.e., underlying nervous and connective tissue elements) might influence the responses. In the present study, to simplify the experimental system, we have taken advantage of the fact that viable ciliated cells exfoliate from the epithelium in large numbers in simple solutions and can be studied independent of the underlying components. Reducing the epithelium to a mixed population of individual ciliated and nonciliated cells that can potentially be isolated and cultured has obvious additional advantages for study which we hope to exploit in the future.

In the isolation media used here, about half the ciliated cells are beating. This percentage remains reasonably constant among different mussels, and duplicate counts taken over several hours remain stable. We cannot yet say what distinguishes beating from nonbeating cells, but L, LF, and F cell populations all distribute themselves similarly. Inasmuch as the gill cells are probably coupled by cell junctions through which small molecules may pass (cf. reference 21), the closure of the junctions at exfoliation is probably crucial to continued ciliary activity and to cell viability. We have bypassed the problem of the large nonmotile cell population for the moment by basing our results on changes in the motile population alone. The fine structure of these cells and of their cilia seems normal and the cell membrane appears intact.

Our experiments clearly demonstrate that ciliary arrest is a property of the gill cells themselves. In contrast to earlier experiments with the excised gill, however, we find that not only are L cells

TABLE IV

Reactivation of Detergent Extracted Cell Models of Ciliated Gill Cells of Elliptio

Cell type	% Motility in	
	Isolation medium A	Reactivation solution
L	51 ± 4.12	42 ± 3.9
LF	41 ± 19.6	43 ± 18.6
F	62 ± 6.9	55 ± 4.4

Each value ± SD represents 16 experiments.

TABLE V

Determination of Critical Ca^{+2} Concentration in Ciliated Cell Models from Two Mussel Gills

	% Motility											
	Medium alone			+4 × 10^{-8} M Ca^{+2}			+8 × 10^{-7} M Ca^{+2}			+10^{-5} M Ca^{+2}		
Cell model type	L	LF	F	L	LF	F	L	LF	F	L	LF	F
Mussel 1	39	42	60	42	50	66	8	31	58	0	45	25
Mussel 2	30	53	66	42	52	75	3	52	64	0	54	60

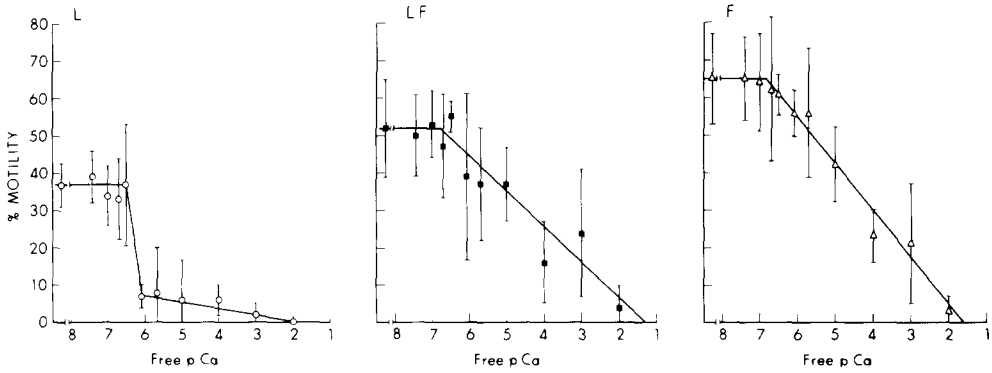

FIGURE 5 Titration curves of motility vs. Ca^{+2} concentration for models of the three gill cell types. Ca^{+2} concentration is plotted logarithmically as pCa (see text). The point closest to the ordinate represents percent motility in reactivation solutions containing no added Ca^{+2}. Note that LF and F cells behave similarly in that there is a gradual loss of model motility with increasing Ca^{+2}; L cells show a different, sharper response to changes in Ca^{+2} between 10^{-7} and 10^{-6} M.

arrested in the presence of A23187 and Ca^{+2}, but that LF and F cells are also affected, although perhaps to a lesser extent.

The results of these ionophore experiments are simply interpreted in terms of the experiments with the gill cell models, while both sets of experiments are completely consistent with the previous hypothesis that increasing axonemal Ca^{+2} is responsible for ciliary arrest in this system. If this is accepted, comparison of Table II with Fig. 5 provides information as to approximately what Ca^{+2} concentration is present in the cells after the application of ionophore in the presence of 12.5 mM external Ca^{+2}. For example, if internal free Ca^{+2} were to rise to $\sim 10^{-4}$ M under the conditions of our ionophore experiments, from Fig. 5 we would expect 7 ± 5% of L cells, 17 ± 12% of LF cells, and 23 ± 7% of F cells to remain motile. Such numbers are clearly compatible with those determined in Table II. If we assume that, in the more complicated perfusion chamber experiments of Satir (19), the effective rise in Ca^{+2} is, for example an order of magnitude lower (i.e., internal $Ca^{+2} = 10^{-5}$ M), then arrest of L cilia would remain nearly complete, but ~80% of normal LF and F cilia would be seen, a percent motility easily interpreted to represent no effect. In addition, Fig. 5 suggests that a physiological change in free Ca^{+2} over relatively narrow limits from ca. 5×10^{-7} M to 8×10^{-7} M would be sufficient to produce differential massive arrest of L-cilia, with LF or F cilia minimally affected. Similar concentrations of Ca^{+2} also arrest *Mytilus* L cilia models (28). On a per cilium basis, this is a change of remarkably few ions. Reversibility would require that Ca^{+2} pumps in the ciliary membrane, the necklace region or elsewhere remove these ions from the axoneme in the course of seconds, which does not seem an improbable rate. The free Ca^{+2} concentration of the axoneme calculated from Fig. 5 falls within the range measured for other cells (1, 2).

We have as yet no explanation of why L cilia are differentially affected by Ca^{+2}. We consider two possibilities most likely, either (*a*) the L cilia contain an additional component, e.g., a Ca^{+2} binding protein, or more of that component than do the other cilia, or (*b*) localization and control of sliding perhaps with regard to coordination of activity is more stringent in the L cilia.

The model experiments (Figs. 4 and 5) support the contention that the site that is sensitive to changes in free Ca^{+2} concentration is part of the ciliary axoneme or the basal apparatus. The remainder of the cell is disrupted in the models and missing more or less completely in the "eyelashes." The ciliary membrane is also disrupted or missing (Fig. 3). Since the models preserve the differential sensitivity of the L cilia found in the intact gill, the difference in the response of L cilia is not due to variation in Ca^{+2} permeability properties of the membranes of the three cell types. We have not rigorously ruled out the possibility that, during model production, more native membrane on average remains associated with the F and LF cilia than with the L cilia, but we consider this less likely than the other possibilities mentioned.

We have demonstrated here that ionic controls similar to those responsible for protozoan ciliary behavior are also responsible for ciliary arrest in a well-defined metazoan ciliated epithelium. As exemplified here, with the model approach, we can begin to sort out those problems dealing with the Ca^{+2} influx itself, i.e., cell membrane properties responsible for the transient and reversible nature of the physiological response, from those concerned directly with the mechanism of axonemal arrest by Ca^{+2}. An important further step in explaining ciliary behavior in general, as well as differential sensitivity of the gill cilia, would be to localize the Ca^{+2} control site that produces arrest precisely within the ciliary axoneme.

In the sliding microtubule model of ciliary activity as presently conceived, the dynein-tubulin interaction is directly responsible for sliding of the doublets (24), whereas the shear resistances which convert sliding into bending are provided by other structures, notably the radial-spoke, central sheath interaction (30). Calcium could control microtubule sliding directly or affect one or another of the systems involved in sliding control (22) or the sliding-bending conversion. Preliminary evidence from the variety of effects on protozoan motility produced by Ca^{+2} suggests that the latter are more likely possibilities (see also reference 16). We are presently studying this experimentally in some detail.

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