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Calcium and the Activity of Cilia on the Gill of the Mussel, *Mytilus edulis**

With 3 Text-figures

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ABSTRACT The effect of reduction of [Ca] in the external solution on the activity of the lateral cilia of *Mytilus* gill was investigated. A sudden reduction of [Ca] from the normal (10 mM) to below about 0.1 mM elicited a brief stoppage of the cilia, while in the solution containing 1 mM Ca^{++} repetitive, rhythmic stoppages were observed. Stimulation with a K-rich solution caused a stoppage response only in the presence of Ca^{++} above about 0.1 mM. Increase in the Mg^{++} concentration in the solution did not compensate for the Ca^{++} deficiency in these effects. In the Ca-free medium, the cilia became to beat faster and synchronously with each other instead of showing a metachronism. Similar effects were observed with the solution containing 1mM EGTA.

The lateral cilia on the gill filament of pelecypod molluscs exhibit two kinds of mechanical responses: one characterized by an increase in their frequency of beat and the other by an abrupt stoppage. Aiello and Guideri (1964) found that stimulation of the visceral ganglion or the branchial nerve with repetitive electrical pulses causes an increase in the frequency of beat of the lateral cilia and suggested that the frequency of beat of these cilia is under the nervous control. On the other hand, spontaneous abrupt stoppages of the lateral cilia have been observed by several investigators (Lucas, 1931, 1932; Nelson, 1951; Dral, 1967), and Takahashi and Murakami (1968) have reported that the stoppage can be brought about by a single electrical pulse applied to the visceral ganglion or the branchial nerve.

There are certain indications that the stoppage response is linked in some way or other to the possible excitable properties of the ciliated cell membrane. Thus, Segerdahl (1922) and Murakami (1968) reported that electric stimulation of the isolated gill filament causes a stoppage response if the electric current flows across the ciliated surface of the lateral cell from inside to outside. Furthermore, the abrupt stoppage can be induced when the gill filament is stimulated with high concentrations of potassium ions (Takahashi, 1971; Takahashi and Tsuchiya, 1971).

* This paper is dedicated to Professor Haruo Kinosita on the occasion of his sixtieth birthday.

These observations lead to an interesting comparison between the stoppage response of the molluscan gill cilia and the so-called ciliary reversal of certain protozoans. There seem to exist several points of similarity between the ciliary responses of these widely separated animal groups. Thus, for instance, it was demonstrated by many authors (Kamada, 1931, 1940; Kinosita, 1936; Naitoh, 1958) that the ciliary reversal is elicited on the cathodal side of the unicellular organism stimulated with a direct current, and, in *Paramecium*, a temporary reversal response is induced by a modification of the potassium ion concentration of the surrounding medium (Kamada and Kinosita, 1940). In *Opalina*, Kinosita (1954) showed that the application of 125 mM KCl gives rise to a temporary reversal of the ciliary beat which is accompanied by a sudden decrease of the transmembrane potential.

On the other hand, the importance of calcium ions to the ciliary reversal has frequently been emphasized (Kamada, 1938, 1940; Jahn, 1962), and Naitoh (1968) put forward a hypothesis that the ciliary reversal response of *Paramecium* occurs when the calcium ions bound by a cellular cation exchange system are liberated in exchange for the externally applied cations other than calcium.

In view of the above facts and also in view of the general importance of calcium ions in many excitatory processes, it is of special interest to study the effect of the calcium ion concentration on the responses of the pelecypod gill cilia. In the present paper a brief survey was made of the effects of calcium on the activity—the stoppage response, the frequency of beat and the metachronal co-ordination—of the lateral cilia on the gill of the mussel, *Mytilus edulis*.

MATERIAL AND METHODS

The material used and the methods of the perfusion experiments and the recording were as described by Takahashi and Tsuchiya (1971). A single gill filament isolated from the ctenidium of *Mytilus edulis* was held across a perfusion chamber through which a steady flow of the bathing solution was maintained at a constant rate of about 3 mm/sec. A Teflon-lined sliding device (Takahashi and Tsuchiya, 1972, fig. 2) ensured a rapid change from one solution to another without any appreciable mechanical disturbance. The activity of the lateral cilia was continuously photographed with a slit camera, and from the record obtained such parameters of ciliary activity as the beat frequency, the wavelength and velocity of metachronal waves, and the duration of stoppage responses, if any, were determined. The beat frequency was also monitored photoelectrically during each experiment by means of a photomultiplier tube and a universal frequency counter.

The normal artificial sea water had the following composition: NaCl, 434 mM; KCl, 10 mM; $CaCl_2$, 10 mM; $MgCl_2$, 53 mM and the pH was adjusted to 8.0 by the addition of $NaHCO_3$. Unless otherwise mentioned, the Ca-free medium and the medium containing 80 mM KCl, which was used as the stimulus, were obtained by omitting the $CaCl_2$ or increasing the KCl in the normal artificial sea water respec-

tively with simultaneous modifications of the NaCl content to keep the total osmolarity of the media unchanged. The media containing reduced concentrations of Ca^{++} were obtained by mixing appropriate proportions of the normal artificial sea water and the Ca-free medium. In most of the experiments, the lateral cilia were activated by 10^{-7} M 5-HT in the bathing solution.

Results

Effects of lowering the Ca^{++} concentration on the ciliary activity

When the solution bathing the gill filament was changed from the normal artificial sea water to the one in which the concentration of Ca^{++} was reduced to 1 mM, the lateral cilia repeatedly showed brief stoppage responses (Fig. 1). The first of such stoppages took place immediately after the change of the solution and the repetition of the responses continued with fairly regular intervals of about 2–3 sec. Each stoppage had the duration of 0.3–0.5 sec and occurred almost simultaneously along the length of the filament within the field of view of the microscope. When the medium was brought back to the normal artificial sea water, the responses ceased immediately. On the other hand, if the medium was changed from the normal artificial sea water to the one with the Ca^{++} concentration lower than 1 mM, i.e., 0.1 mM, 0.01 mM, or 0 mM (Ca-free), the stoppage response occurred only once or twice, immediately after the change of the solution. No further stoppages were observed in these very low concentrations of Ca^{++} after this initial response and also when, after 30 sec, the solution was changed again to the normal artificial sea water.

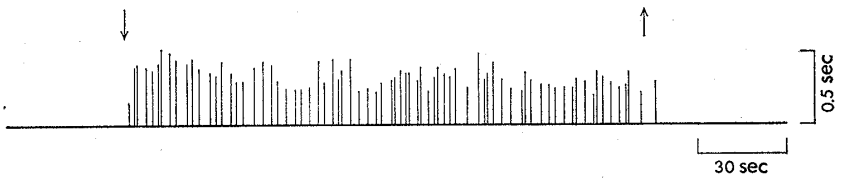

Fig. 1. Repetitive stoppage responses caused by the solution containing 1 mM Ca^{++}. Each stoppage is represented by a vertical bar with the length corresponding to the duration of the response. The arrows indicate the beginning and the end of application of the test solution. Temperature, 27°C.

The frequency of beat was also affected by the concentration of Ca^{++}. Within a minute after the change from the normal artificial sea water to the Ca-free medium, the frequency began to rise rapidly and attained a steady level within 5 minutes. A further addition of 1 mM EGTA (ethyleneglycol bis (amino-ethylether)-N,-N′-tetra-acetic acid) to the medium had no appreciable effect on the result (Fig. 2). The cilia continued to beat at this increased frequency for more than an hour. Figure 2 also shows the changes in the wavelength and the velocity of the metachronism. It will be seen that, in the Ca-free medium, the wavelength increased to

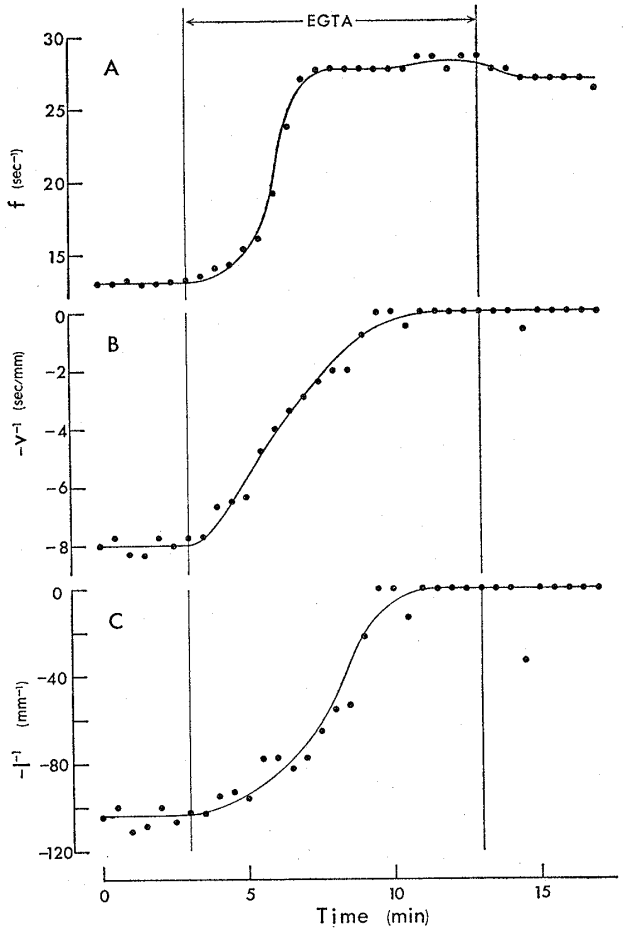

Fig. 2. Effect of Ca-free medium containing 1 mM EGTA on the frequency of beat (A), the wave velocity (B) and the wavelength (C) of the metachronal waves. The wave velocity and the wavelength are plotted as negative reciprocals to avoid infinity. Thus, the zero values on the ordinates in B and C indicate synchronous beatings of the cilia. f, frequency; v, wave velocity; l, wavelength. Temperature, 24°C.

infinity, indicating that the cilia exhibited synchronism instead of metachronism. It will also be noted that the wavelength and the wave velocity still continued to increase when the frequency of beat has already reached the plateau. The medium containing 0.1 mM Ca^{++} or 0.01 mM Ca^{++} increased the frequency of beat like the Ca-free medium, while the medium containing 1 mM Ca^{++} had little effect on the frequency of beat.

Effects of lowered Ca^{++} concentrations on the stoppage response caused by K^{+} stimulation

The abrupt stoppage responses of the lateral cilia caused by high concentrations of K^{+} (Takahashi, 1971; Takahashi and Tsuchiya, 1971), were lost when the concentration of Ca^{++} outside the gill filament was reduced beyond a certain level.

In these experiments, the gill filament was first perfused for 5 min with a medium with a reduced concentration of Ca^{++} (the adaptation medium) and then stimulated with the medium containing 80 mM K^+ (the stimulation medium). If the Ca^{++} concentration in the stimulation medium was the same as in the adaptation medium, the cilia did not respond with a stoppage to 80 mM K both in the medium containing 0.01 mM Ca^{++} and in the Ca-free medium. In the medium containing 0.1 mM Ca^{++}, the response was elicited in one out of four preparations tested, but the duration of the response was much shorter than normal. The cilia apparently responded with a stoppage or stoppages to 80 mM K^+ in the presence of 1 mM Ca^{++}. However, since the adaptation medium containing 1 mM Ca^{++} caused repetitive stoppages of the lateral cilia as has already been described (see Fig. 1 above), it was not always easy to distinguish the response to K^+ from the stoppages caused by the adaptation medium. Nevertheless, examination of the slit-camera records revealed some instances where a distinct responses were elicited by the stimulation medium. In such cases, the duration of the stoppage was usually longer than that of the stoppage elicited in lower concentrations of Ca^{++}. In Fig. 3, the mean duration of the stoppage responses are plotted against the Ca^{++} concentration in the medium. Experiments which were the same as above except that the amount of $MgCl_2$ and not of NaCl was increased to compensate for the osmotic change due to the reduction of $CaCl_2$ gave results which were not different from those of the above experiments.

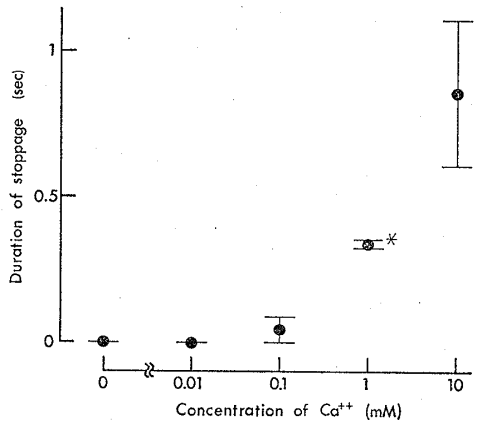

Fig. 3. Effects of Ca^{++} concentration in the medium on the duration of the stoppage response caused by a high concentration of K^+(80 mM). Plotted as the mean durations of the stoppages with the standard errors. *Some of the stoppages caused by the medium containing 80 mM K^+ and 1 mM Ca^{++} were difficult to distinguish from the 'spontaneous' stoppages caused by the adaptation medium containing 1 mM Ca^{++} (see text). The duration of the stoppages, however, did not seem to differ significantly between these two responses. Temperature, 24°C.

Although the cilia did not respond to 80 mM K^+ if Ca^{++} was absent from both the adaptation and the stimulating media, they responded with an abrupt stoppage when stimulated with a medium containing 10 mM Ca^{++} as well as 80 mM

K^+ even after a 5 min period of adaptation in the Ca-free artificial sea water. Similarly, the Ca-free stimulating medium containing 80 mM K^+ elicited a brief stoppage response if Ca^{++} (10 mM) was present in the adaptation medium. However, no response was observed when the preparation which had been in the Ca-free medium containing 80 mM K^+ was exposed to a medium containing 10 mM Ca^{++} as well as 80 mM K^+.

DISCUSSION

The similarity that exists between the abrupt stoppage response of the lateral cilia and the ciliary reversal response of the ciliate protozoans have already been pointed out (p. 64). The finding of the present study that the cilia previously treated with the Ca-free medium, or with the medium containing a very low concentration of Ca^{++}, failed to respond to stimulation with a high concentration of K^+ presents another similarity between these two responses. Thus, in *Opalina*, Kinosita reported (1954) that, while the application of a KCl solution after the previous treatment of the organism with Ringer's solution always gave rise to a sudden depolarization with a transient overshooting of the membrane potential, neither the overshooting nor the ciliary reversal was observed when the KCl solution was applied after the previous treatment of the organism with the Ca-free Ringer's solution.

The role played by the calcium ions in the stoppage response is unknown. However, the fact that the response can be elicited when the gill filament previously treated with the Ca-free adaptation medium was stimulated with the K^+-rich medium which contained 10 mM Ca^{++}, suggests that the Ca^{++} in the external medium can be rapidly utilized for the response. The fact that the application of the solution containing 80 mM K^+ as well as 10 mM Ca^{++} after the previous treatment with the solution containing 80 mM K^+ without Ca^{++} did not cause the response may indicate that, for the stoppage response, Ca^{++} is required at the moment of transition from the normal to the high K^+ concentration and that the cilia previously treated with the high concentration of K^+ lose the ability for the response. This may be in agreement with the finding of Murakami (1968) that the cilia previously treated with a high concentration of K^+ could not be induced to stop by electrical stimulation.

The present experiments have shown that the stoppage response can be elicited by a sudden reduction of the Ca^{++} concentration in the artificial sea water. It is difficult to explain the mechanism of this response. Naitoh (1968) suggested that the reversal response of *Paramecium* occurs when Ca^{++} bound by an cation exchange system are liberated. A similar mechanism may account for the stoppage responsee caused by a decrease in the concentration of Ca^{++} in the external medium.

The result that an increase of Mg^{++} concentration in the Ca^{++}-free medium did not restore the stoppage response indicates that Mg^{++} does not act as a substitute

for Ca^{++} in the stoppage response.

The repetitive excitation caused by a low Ca^{++} concentration is well known in the muscle and the nerve. For example, Bülbring *et al.* (1956) showed that a curarized skeletal muscle of frog becomes rhythmically active, namely, the membrane potential becomes unstable and gives rise to spontaneous action potentials which result in repeated twitches, when the Ca^{++} concentration in the medium is reduced to one twentieth of that in the normal solution. The repetitive stoppages observed in the medium containing 1 mM Ca^{++} may also be attributable to the instability of the ciliated cell membrane. Another possibility is that the deficiency of Ca^{++} in the bathing solution brings about rhythmic discharges of nerve fibres in the gill filament, resulting in the repeated stoppages of the lateral cilia. The presence of such nerve fibres have been inferred by Takahashi and Murakami (1968).

Similarly, the results that the frequency of beat was increased by the Ca-free medium may be explained by the activity of the 'cilioexcitatory' nerve fibers (Aiello and Guideri, 1964). However, according to Aiello (1960), the high frequency of beat (22.5–25.1 beats per sec) caused by 5-HT (2×10^{-5} M), the possible mediator of the action of the cilioexcitatory nerve, was not accompanied by an increased wavelength, whereas in the present experiment the wavelength increased to infinity (synchronization) as the frequency of beat increased (to about 28 beats per sec). The difference between the effects of 5-HT and of the Ca-free medium indicates that the latter effect may not be attributable solely to the activation of cilioexcitatory nerve. In view of the ciliary co-ordination, it is interesting that the wavelength as well as the wave velocity continued to increase when the frequency of beat has already reached the plateau and that the cilia finally exhibited a synchronism. Considerable knowledge concerning the metachronism of the lateral cilia has been accumulated by the works of Gray (1930), Lucas (1932) and Aiello (1960), but the synchronism of these cilia as seen in this experiment or in the preceding paper (Takahashi and Tsuchiya, 1971) in which the synchronism caused by high concentrations of K^+ was described has never been observed. The present results suggest that the mode of co-ordination of the lateral cilia depends on such conditions of the cilia or the ciliated cell that can be modified by the concentration of Ca^{++} or K^+ in the external medium.

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