

## SODIUM–CALCIUM ACTION POTENTIAL ASSOCIATED WITH CONTRACTION IN THE HELIOZOAN *ACTINOCORYNE CONTRACTILIS*

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### SUMMARY

The electrophysiology of the contractile protozoan *Actinocoryne contractilis* was studied with conventional intracellular recording techniques.

Resting membrane potential (−78 mV, s.d. = 8,  $N = 18$ ) was dependent upon external  $K^+$ . Rapid action potentials (overshoot up to 50 mV) were evoked either by mechanical stimulation or by current injection. Graded membrane depolarizations induced by graded mechanical stimuli correspond to receptor potentials.

The receptor potential was mainly  $Na^+$ -dependent; the action potential was also mainly  $Na^+$ -dependent, but involved a minor  $Ca^{2+}$ -dependence. The two components of the action potential could be separated in  $Ca^{2+}$ -free solution containing EGTA ( $1\text{ mmol l}^{-1}$ ), in low- $Na^+$  solutions or by the addition of  $Co^{2+}$ . The repolarizing phase of the action potential was sensitive to TEA ions and to 4-aminopyridine (4-AP).

Action potentials were followed in 10–20 ms by a rapid all-or-none contraction of the axopods and stalk. Contraction was blocked in  $Ca^{2+}$ -free solution containing EGTA and by  $Co^{2+}$ , which suggests a requirement of external  $Ca^{2+}$  for this event. Contraction was also abolished by 4-AP.

### INTRODUCTION

In different protozoans, external stimuli evoke a variety of responses, including escape or avoidance reactions, light emission and cell contraction (Eckert & Sibaoka, 1968; Naitoh, 1968; Wood, 1982). The remarkable stalked marine heliozoan *Actinocoryne contractilis* Febvre-Chevalier, which consists of a head, bearing long axopods, attached to an amoeboid base by a single or multiple stalk (Fig. 1A) is very sensitive to mechanical stimuli. Under normal conditions, it reacts to brief mechanical stimuli or to vibrations by a very rapid (50–60 ms) all-or-none

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contraction of axopods, head and stalk into the base. The axopods appear less sensitive to local mechanical stimuli than the head itself, for example contact of slow-moving prey organisms with an axopod leads to the production of a food vacuole which passes along towards the head; however, extensive stimulation of many axopods elicits contraction of the cell. External electric fields also elicit contraction. The patterns of contraction resulting from electrical and mechanical stimuli have been examined previously (Febvre-Chevalier & Febvre, 1980; Febvre-Chevalier, 1981) using high-speed cinematography ( $8000 \text{ frames s}^{-1}$ ). Contraction can also be evoked by current injection, as described in the present paper.

Re-extension of the head and stalk is a relatively slow process, taking 20 min or so (Febvre-Chevalier & Febvre, 1980; Febvre-Chevalier, 1981). Since the head is up to  $40 \mu\text{m}$  in diameter in large individuals, and microelectrodes may be inserted in it without evoking contraction, *Actinocoryne* offers a peculiarly suitable preparation for examining the correlation of membrane electrical events with contractile activity. In other protozoans studied previously, the relationship between membrane electrical events and contraction is not well understood (see Naitoh, 1982). This paper describes the initiation and ionic basis of the propagated action potential related to contraction.

#### MATERIALS AND METHODS

Specimens of *Actinocoryne* were collected from benthic habitats (see Febvre-Chevalier, 1980) in the bay of Villefranche-sur-Mer (in the Mediterranean near Nice). The heliozoans and the small piece of substrate to which they were attached were kept in filtered sea water in Petri dishes, and fed upon oligotrich ciliates. One or several specimens were transferred into solid watchglasses containing about 5 ml of solution for the experiments. Mechanical stimuli were delivered either by simply tapping the mounting of the recording electrode (in conjunction with a transient store with pre-triggering this gave records of potential changes prior to retraction of the cell) or with a blunted glass micropipette attached to a small speaker. Graded mechanical stimuli of 1–5 ms duration given by the latter method were approximately proportional to the speaker coil current, but for various reasons were only semi-quantitative. Electrical stimuli were given *via* a second electrode inserted in the head (Fig. 1B) after placement of the recording electrode; the current injected was measured across a  $10\text{-M}\Omega$  resistor in the ground line. Both electrodes were filled with KCl ( $3 \text{ mol l}^{-1}$ ) and had resistances between 8 and  $10 \text{ M}\Omega$ . Contractile activity was not monitored, although the delay between the membrane response and the beginning of contraction was approximately indicated by the abrupt return to zero potential as the recording electrode was displaced from the cell by the contraction (Fig. 3B,C).

The reference artificial sea water solution (ASW) had the following composition (in  $\text{mmol l}^{-1}$ ): NaCl, 500;  $\text{MgCl}_2$ , 58; KCl, 10;  $\text{CaCl}_2$ , 10; and was buffered to pH 8.0 with tris (hydroxymethyl)-aminomethane-HCl (Tris-HCl) or tris (hydroxymethyl)methylaminopropane sulphonic acid (TAPS).  $\text{Na}^+$ -free solutions were made

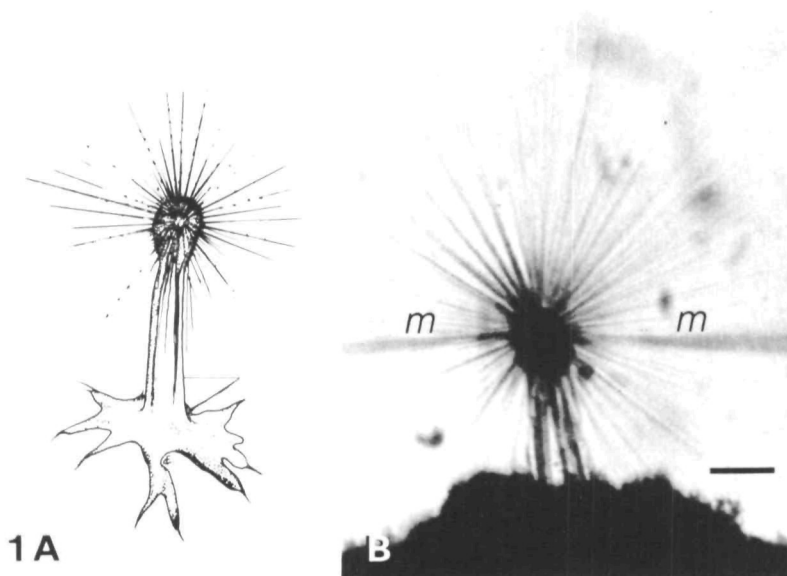


Fig. 1. (A) Schematic drawing of the heliozoan *Actinocoryne contractilis*. A globular head bearing long axopods is attached to an amoeboid base by a single or multiple (not shown) stalk. Unless otherwise indicated in the text, both recording and stimulating microelectrodes were placed in the head of the cell. (B) Animal attached to substrate impaled by two microelectrodes (*m*) placed in the head.

by substituting NaCl with Tris or occasionally with *N*-methyl glucamine. Tris-glycine and choline chloride were not found suitable.  $\text{Ca}^{2+}$ -free solutions were made by omitting  $\text{CaCl}_2$  and adding  $0.5\text{--}1.0\text{ mmol l}^{-1}$  ethyleneglycol-bis-( $\beta$ -aminoethylether)*N,N'*-tetra-acetic acid (EGTA). Variations from ASW up to  $50\text{ mmol l}^{-1}$  were not corrected osmotically.

$\text{Co}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Sr}^{2+}$  and  $\text{Ba}^{2+}$  were used at  $40\text{--}50\text{ mmol l}^{-1}$ . 4-Aminopyridine (4-AP) was used at concentrations between 1 and  $10\text{ mmol l}^{-1}$ . pH of the solution was adjusted to that of sea water prior to experiments. Tetraethylammonium chloride (TEA) was used at  $10\text{ mmol l}^{-1}$ .

Solutions were exchanged using a peristaltic pump with a flow rate of  $3\text{--}4\text{ ml min}^{-1}$ . The animals were incubated in test solutions for a minimum of 10 min prior to experiment. All experiments were performed at room temperature ( $10\text{--}20^\circ\text{C}$ ).

## RESULTS

### *Resting potential*

The resting potential was remarkably stable, and did not alter even if some axopods contracted. Maximum and minimum values were  $-86$  and  $-68\text{ mV}$  ( $-78\text{ mV}$ , S.D. = 8,  $N = 18$ ). The membrane potential approximated to the

behaviour of a Nernst electrode (Fig. 2), although measurements could not be made at high external  $K^+$  concentrations as the cells contracted.

### *Receptor potential*

A series of mechanical stimuli of increasing strength evoked a series of graded membrane depolarizations, either followed by slower repolarizations (Fig. 3A) or if the threshold value around  $-20$  mV was reached, by an overshooting action potential (Fig. 3B). Subthreshold graded depolarizations summated to produce an action potential provided they were less than 20 ms apart (Fig. 3C). In view of the graded response to increasing stimuli and the way in which they may summate, these depolarizing potentials are regarded as receptor potentials. The receptor potentials were of characteristic form, showing a rapid rise and initial fall, followed by a slower repolarization phase restoring resting potential after some 300 ms. In low- $Na^+$  or  $Na^+$ -free solutions, receptor potentials large enough to generate action potentials could not be produced by mechanical stimuli that were large enough to evoke action potentials in ASW (Fig. 4A,B); but were seen when external  $Na^+$  was increased. The amplitude of the receptor potentials evoked was correlated with external  $Na^+$  concentration, but the relationship between values for peak receptor potential and external  $Na^+$  has not been determined. Receptor potentials apparently identical to

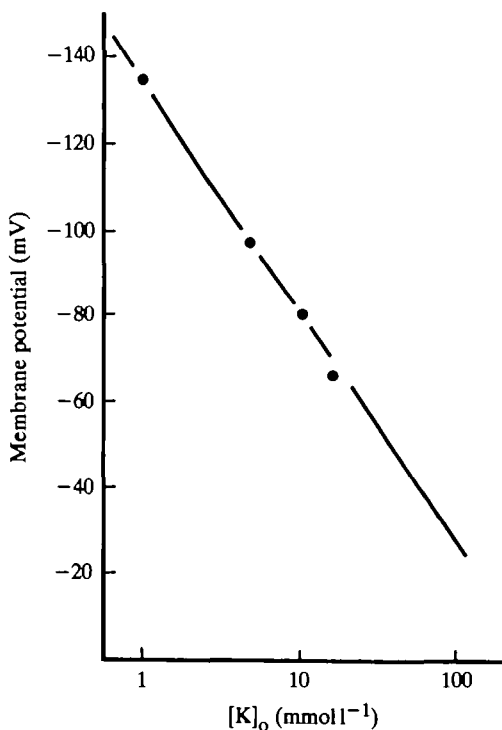


Fig. 2. Semi-logarithmic plot showing the potassium dependence of the resting potential. Data from several cells.

those in ASW were observed when animals were mechanically stimulated in  $\text{Ca}^{2+}$ -free solutions, but a small contribution of  $\text{Ca}^{2+}$  to the receptor potential has not been excluded.

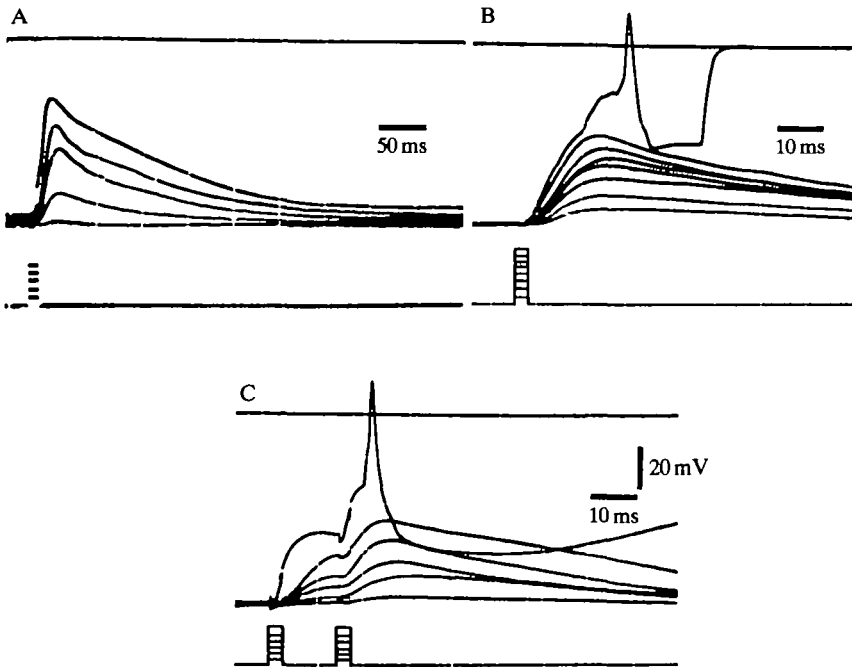


Fig. 3. Receptor potentials evoked by mechanical stimulation of the cell. Mechanical shocks of increasing intensity (bottom line retouched) produced graded membrane responses (A) which gave rise to an action potential (B). In B, the repolarizing phase of the action potential is interrupted by the rapid return of the electrode potential to zero potential. This results from the contraction of the cell with consequent withdrawal of the head from the recording electrode. The beginning of the return to zero potential gives an indication of the delay between the action potential and the onset of contraction. (C) Shows the summation of receptor potentials which also elicited a spike. In this record, the contraction of the cell was delayed and was fully achieved off the recording. Upper trace, zero potential.

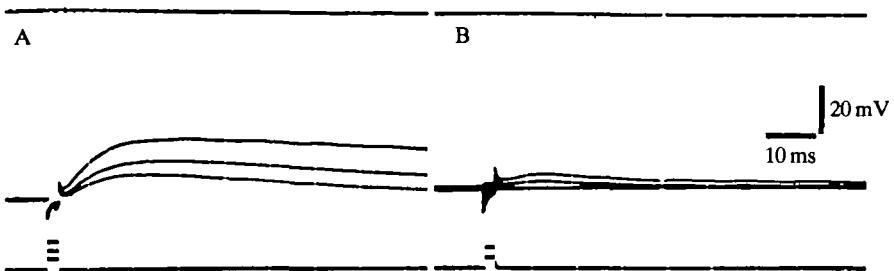


Fig. 4. Receptor potentials evoked by strong mechanical stimuli applied to cells incubated in low sodium solution ( $100 \text{ mmol l}^{-1} \text{ Na}^+$  in A;  $10 \text{ mmol l}^{-1} \text{ Na}^+$  in B). The amplitude of the receptor potentials never reached the firing threshold for spike generation. Upper trace, zero potential.

*Action potential*

Action potentials were evoked by current injection (Fig. 5). They were identical to action potentials produced by mechanical stimulation (Fig. 3B). The firing threshold of action potentials evoked by current injection was between 58 and 75 mV above the resting potential. Around these values, the input resistance of the cell decreased. The  $I/V$  relationship was linear over the range of  $-80$  mV to  $+60$  mV (resting potential = 0) (Fig. 6). Above 60 mV, the slope changed indicating outward rectification, and action potentials could be evoked by an increase in injected current. The apparent input resistance of the cell over the range below action potential threshold was around  $100\text{--}400\text{ K}\Omega$ . The amplitude of electrically evoked action potentials varied between 110 and 134 mV (121 mV, s.d. = 9,  $N = 18$ ), and overshoots were between 28 and 50 mV (42.5 mV, s.d. = 8,  $N = 18$ ). Action potential duration at half spike amplitude was 0.5 ms, and spike origin to return to resting potential occupied around 10 ms. There was no undershoot.

It was sometimes possible to insert an electrode into the base of an animal which was contracted, in which case identical action potentials were recorded on mechanical stimulation (by tapping the electrode) to those seen in the head of expanded animals (Fig. 7A), except that since there was no further contraction, the electrode remained in the cell. Simultaneous records from electrodes placed in the head and base of specimens attached to glass coverslips showed that mechanical stimuli to the head evoked action potentials that were followed by similar, though somewhat attenuated, potential changes in the base (Fig. 7B). Since normal action potentials could be recorded from the base of contracted specimens, it is probable that the attenuation resulted from the difficulty of recording from the very thin base of the animals attached to coverslips and that the membrane of the entire cell is excitable and propagates action potentials. If this is the case, they are propagated at around  $3\text{ ms}^{-1}$ .

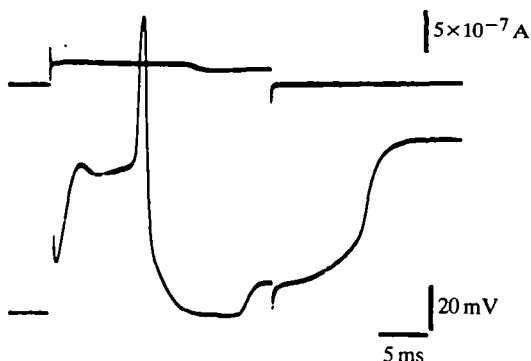


Fig. 5. Membrane response evoked by electrical stimulation. An action potential (lower trace) was produced in response to a current pulse (upper trace) applied intracellularly. Subsequent to the spike, the electrode potential returns to zero potential giving evidence of the contractile activity of the cell.

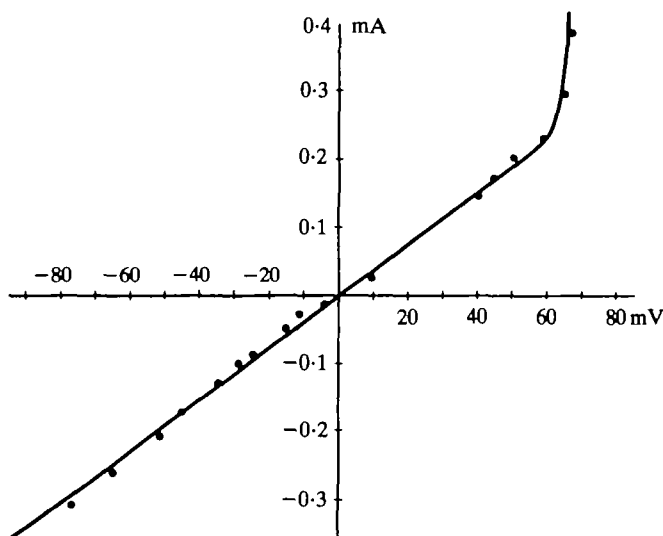


Fig. 6. Plot showing I-V relationship. Voltage was measured at the end of a 30-ms pulse. The curve obtained for this cell closely resembled that from two other cells examined.

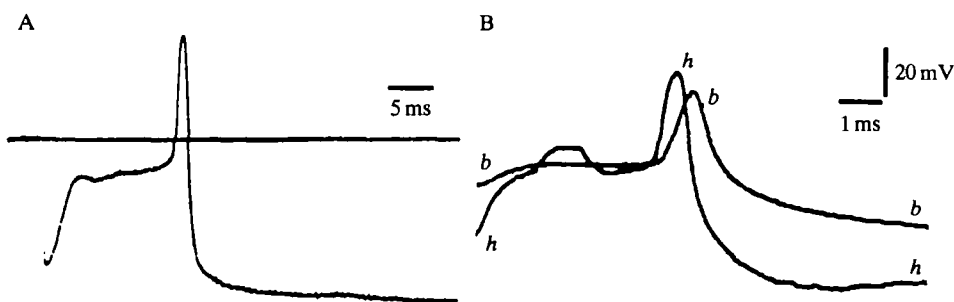


Fig. 7. (A) Action potential recorded in the flat base of the cell. No contractile activity followed the membrane response which was evoked by mechanical stimulation. (B) Propagation of action potentials from the head (lower trace, *h*) towards the base (upper trace, *b*) of the cell. Resting potential in the flat base was incompletely established. Mechanical stimulation *via* electrode in head.

### *The ionic basis of the action potential*

Action potentials could be evoked in  $\text{Ca}^{2+}$ -free solutions (Fig. 8A) and in  $\text{Na}^{+}$ -free solutions (Fig. 8B).

### *$\text{Na}^{+}$ -dependence of the action potentials*

In SW or ASW solutions containing tetrodotoxin (TTX) at concentrations between  $10^{-9}$  and  $10^{-6}$  mmol l $^{-1}$  the action potential was unaffected, and normal rapid contraction followed the action potential.

In  $\text{Ca}^{2+}$ -free ASW with 1 mmol l $^{-1}$  EGTA, action potentials resembled those in SW or ASW, but were not followed by contraction (Fig. 8A). In such a  $\text{Ca}^{2+}$ -free solution, resting membrane potential approached that observed in ASW

( $-74$  instead of  $-78$  mV). In contrast, the firing threshold and the overshoot decreased,  $-30$  mV instead of  $-20$  mV and  $27.5$  mV instead of  $42.5$  mV, respectively.

Below  $150 \text{ mmol l}^{-1} \text{ Na}^+$  action potentials could not be evoked in  $\text{Ca}^{2+}$ -free solutions, even with large depolarizations; above  $150 \text{ mmol l}^{-1} \text{ Na}^+$  the amplitude of the overshoot was related to  $\text{Na}^+$  concentration, the slope being around  $30$  mV per decade change in  $\text{Na}^+$  instead of  $58$  mV as predicted by the Nernst equation (Fig. 9). Sometimes, in  $\text{Ca}^{2+}$ -free solutions, rhythmic firing occurred a few seconds after impalement (Fig. 10) following initial large oscillations of membrane potential which led to a series of 10 or more action potentials at  $0.5$ – $1.0$  Hz.

Contractile activity is evidently critically dependent upon external  $\text{Ca}^{2+}$  concentration. In  $\text{Ca}^{2+}$ -free solutions containing less than  $1 \text{ mmol l}^{-1}$  EGTA, rapid contraction followed the action potential, although after prolonged sojourn in such solutions, and several cycles of contraction and re-extension, contractile activity following subsequent action potentials was much slowed or even abolished. With  $1 \text{ mmol l}^{-1}$  or more EGTA, contractile activity was completely abolished from the outset in the majority of experiments (Figs 8A, 10). In some cases, however, a slow contraction of the stalk followed one or more action potentials, this kind of slow contraction being entirely different to the normal rapid contraction. If such slow contractions occurred, the stalk and head were subsequently re-extended in the same  $\text{Ca}^{2+}$ -free solution with EGTA.

Addition of  $10$ – $50 \text{ mmol l}^{-1} \text{ Mn}^{2+}$  to ASW had no obvious effect upon the action potential, but contraction was either delayed or slowed. Addition of  $10$ – $50 \text{ mmol l}^{-1} \text{ Co}^{2+}$  to ASW or SW after a few minutes induced initially a slow contraction of the stalk, which either began spontaneously or when the electrodes were inserted. This slow contractile activity was not accompanied by any changes in membrane potential. In a few experiments addition of  $\text{Co}^{2+}$  solutions did not cause contraction of the stalk, and in such cases, repetitive action potentials were evoked during current injection. These action potentials were not followed by contraction (Fig. 11).

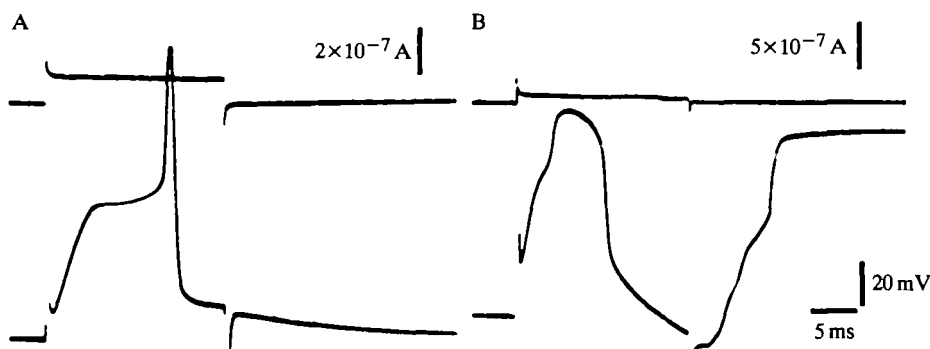


Fig. 8. Membrane responses (lower traces) evoked by current pulses (upper traces, B) applied to cells incubated in either  $\text{Ca}^{2+}$ -free solution containing  $1 \text{ mmol l}^{-1}$  EGTA (A) or  $\text{Na}^+$ -free solution containing  $10 \text{ mmol l}^{-1} \text{ Ca}^{2+}$  ions (B). Both records show that action potentials persist, although in  $\text{Na}^+$ -free solution (B), the plateau potential prolongs the depolarizing phase of the spike. Contractile activity following the spike potential which occurs in  $\text{Na}^+$ -free solution (B) is not observed in  $\text{Ca}^{2+}$ -free solution (A).

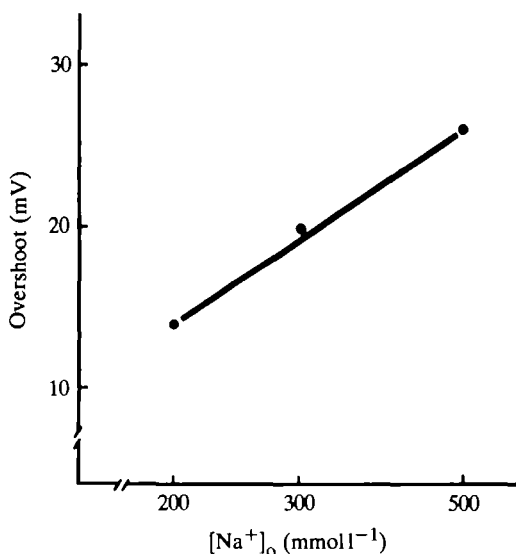


Fig. 9. Variation of the overshooting amplitude of the action potential plotted as a function of logarithmic change of the external sodium concentration ( $Ca^{2+}$ -free solution). The slope is about 30 mV per decade.

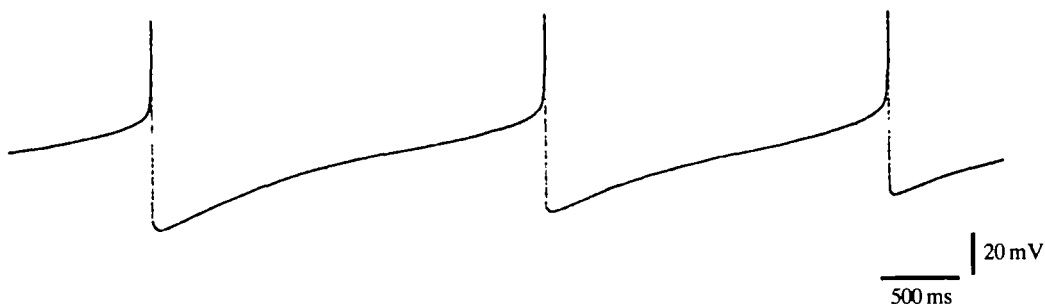


Fig. 10. Repetitive membrane firing recorded in  $Ca^{2+}$ -free solution (EGTA, 1 mmol l<sup>-1</sup>;  $Na^+$ , 500 mmol l<sup>-1</sup>). Rhythmic action potentials were spontaneously produced following large disordered oscillations of the membrane potential.

### *$Ca^{2+}$ -dependence of the action potentials*

In most experiments with  $Na^+$ -free solutions, the axopods retracted soon after the solution was introduced, to form a thick, sticky layer which made impalement difficult. Resting potential ( $-80$  mV) slightly exceeded the mean value in ASW, whatever the  $Ca^{2+}$  content of the solution. Occasionally, long-lasting, irregular oscillations of membrane potential were observed upon impalement; these did not evoke regenerative responses.

No regenerative responses were seen when  $Na^+$ -free solutions contained less than 10 mmol l<sup>-1</sup>  $Ca^{2+}$ . They could be elicited with great difficulty when the  $Ca^{2+}$  content was 10 mmol l<sup>-1</sup> (the level in ASW). In such solutions, the response of the membrane to current injection was often a plateau depolarization whose amplitude and duration increased with that of the injected current. Occasionally, overshooting

action potentials were obtained. Unlike those in ASW, however, the spike was followed by a short (5–8 ms) plateau before rapid repolarization (Fig. 8B).

Regenerative membrane responses were obtained more easily in  $\text{Na}^+$ -free solutions, by increasing  $\text{Ca}^{2+}$  above its normal level in SW or ASW (up to  $200 \text{ mmol l}^{-1}$ ). In such high- $\text{Ca}^{2+}$  solutions, the spike was not followed by a plateau (Fig. 12A,B) and the firing threshold for the action potential rose with increasing

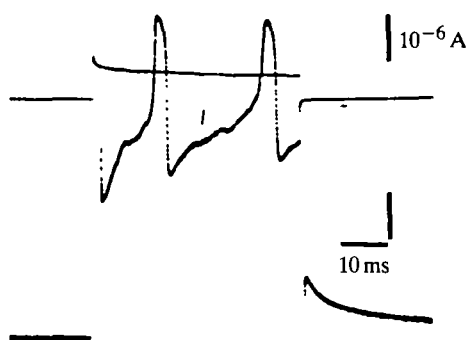


Fig. 11. Membrane activities recorded in the presence of  $\text{Co}^{2+}$  ions ( $20 \text{ mmol l}^{-1}$ ) added to the normal saline. The two action potentials (lower trace) evoked during current injection (upper trace) did not trigger a contractile response.

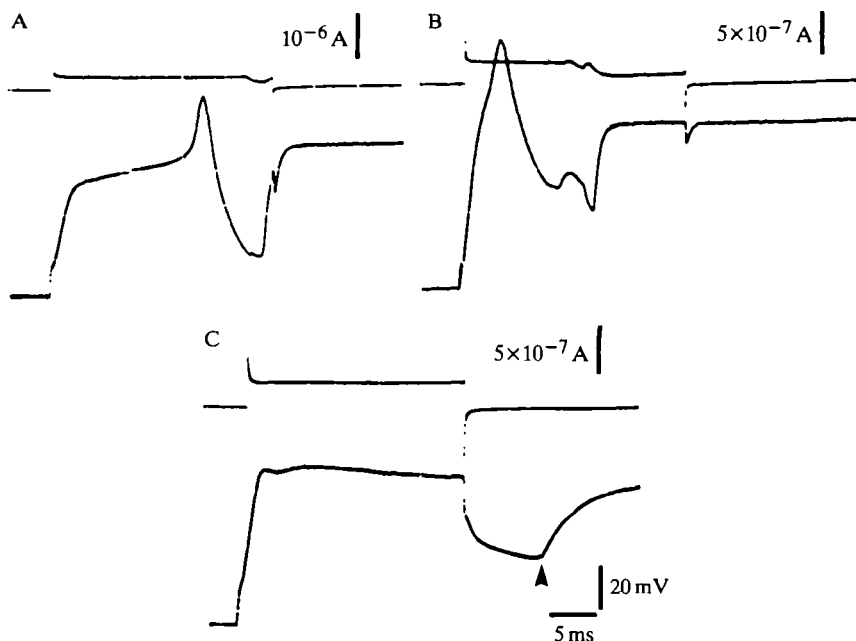


Fig. 12. Membrane responses (lower traces) of cells incubated in  $\text{Na}^+$ -free solutions containing  $\text{Ca}^{2+}$ -ions at concentrations of  $30 \text{ mmol l}^{-1}$  (A) and  $100 \text{ mmol l}^{-1}$  (B). An increase in the amplitude of the spike overshoot clearly appeared at higher  $\text{Ca}^{2+}$  concentrations (B). (C) Although the membrane potential was strongly depolarized by the current pulse, an action potential failed to be produced in  $\text{Na}^+$ -free solution containing  $25 \text{ mmol l}^{-1}$   $\text{Ca}^{2+}$  ions. However, subsequent to the plateau potential, contraction occurred (arrowhead). Upper traces, current monitor.

$\text{Ca}^{2+}$ . The slope of the variation of the overshooting amplitude in such high- $\text{Ca}^{2+}$ ,  $\text{Na}^+$ -free solutions (extrapolated for a decade change in external  $\text{Ca}^{2+}$  concentration) was some 28 mV, close to the value for a  $\text{Ca}^{2+}$  electrode. Action potentials in  $\text{Na}^+$ -free solutions containing 50–200  $\text{mmol l}^{-1}$   $\text{Ca}^{2+}$  were followed by rapid contractions.

Contractile activity in  $\text{Na}^+$ -free solutions had also been observed in preparations incapable of producing regenerative responses (Fig. 12C). Here, the contractions of the stalk were incomplete and slower, and were observed after large depolarizing pulses which took membrane potential close to zero potential. Such contractions have been observed even in solutions containing less than 30  $\text{mmol l}^{-1}$   $\text{Ca}^{2+}$ .

In some preliminary experiments with  $\text{Ba}^{2+}$  and  $\text{Sr}^{2+}$ ,  $\text{Na}^+$ -free solutions were used when  $\text{Ca}^{2+}$  was substituted to avoid  $\text{Na}^+$ -dependent electrogenesis. When  $\text{Ca}^{2+}$  was substituted by 50  $\text{mmol l}^{-1}$   $\text{Ba}^{2+}$ , resting potential remained unchanged at  $-80$  mV but regenerative response and contraction were not observed. Solutions containing 50  $\text{mmol l}^{-1}$   $\text{Sr}^{2+}$  evoked contraction as soon as they came into contact with the cell, usually before it could be impaled. With prior treatment in  $\text{Na}^+$ -free solutions without  $\text{Ca}^{2+}$  and with 1  $\text{mmol l}^{-1}$  EGTA, the cells remained extended when  $\text{Sr}^{2+}$  solution was added. Resting potential was  $-90$  mV, and as with  $\text{Ba}^{2+}$  substitution, no regenerative response was observed. However, in contrast to  $\text{Ba}^{2+}$  solutions, contraction followed injection of depolarizing current pulses. As already mentioned, the  $\text{Ca}^{2+}$  blocking agents  $\text{Co}^{2+}$  and  $\text{Mn}^{2+}$  added to SW or ASW produced no obvious effects upon the action potential, but contractions were slowed or abolished.

### *The repolarizing phase of the action potential*

Both TEA and 4-AP (known as blocking agents for voltage-dependent  $\text{K}^+$  conductances) prolonged the repolarizing phase of the action potential. Addition of 10  $\text{mmol l}^{-1}$  TEA to ASW (Fig. 13A) greatly lengthened the repolarizing phase, and contraction occurred before the resting potential was attained. Similarly, 4-AP at 10  $\text{mmol l}^{-1}$  lengthened the initial repolarizing phase, but this was followed by a more rapid repolarization to resting potential level (Fig. 13B). At 1–2  $\text{mmol l}^{-1}$ , 4-AP did not obviously affect repolarization, but rhythmic action potentials were evoked, initiated either by impalement or by the first current pulse (Fig. 14).

Much the most striking and unexpected effect of 4-AP was that, at concentrations above 1  $\text{mmol l}^{-1}$ , contraction was completely abolished (Fig. 14) and the appearance of the animal changed. The stalk and axopods became rigid, and whereas in SW or ASW, prior to the addition of 4-AP, the axopods could be bent if lightly stroked with a fine probe, after the addition of 4-AP they were so rigid that they could be broken off.

In  $\text{Na}^+$ -free solutions containing 50–100  $\text{mmol l}^{-1}$   $\text{Ca}^{2+}$  and 1–2  $\text{mmol l}^{-1}$  4-AP, action potentials could be generated by current injection. At 50  $\text{mmol l}^{-1}$   $\text{Ca}^{2+}$ , regenerative responses were difficult to obtain, but if evoked, were not followed by contractile activity (Fig. 15A). Above 50  $\text{mmol l}^{-1}$   $\text{Ca}^{2+}$ , regenerative responses were easier to obtain and were then followed by rapid contractions (Fig. 15B).

## DISCUSSION

Membrane electrogenesis has been extensively studied in ciliates (reviewed by Naitoh, 1982); *Actinocoryne* is the first heliozoan examined. In free-swimming

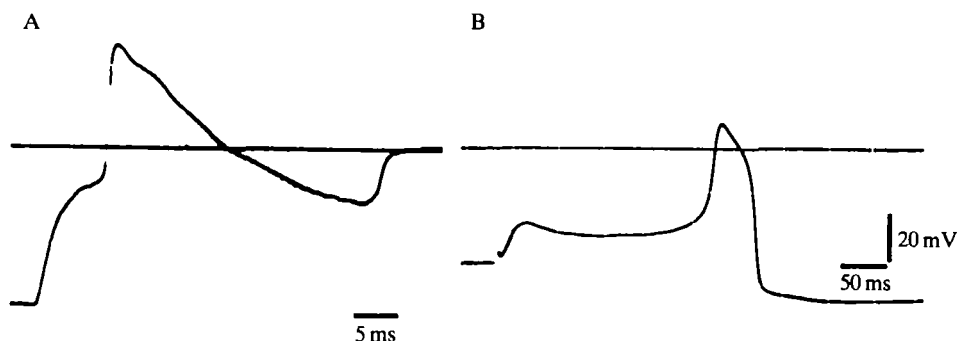


Fig. 13. Effects of tetraethylammonium chloride (TEA) and 4-aminopyridine (4-AP) ( $10 \text{ mmol l}^{-1}$ ). (A) TEA ions prolong the repolarizing phase of the mechanically-evoked action potential. Contraction occurs before the complete repolarization of the membrane potential. (B) 4-AP also prolongs the repolarizing phase, but no contraction follows the action potential.

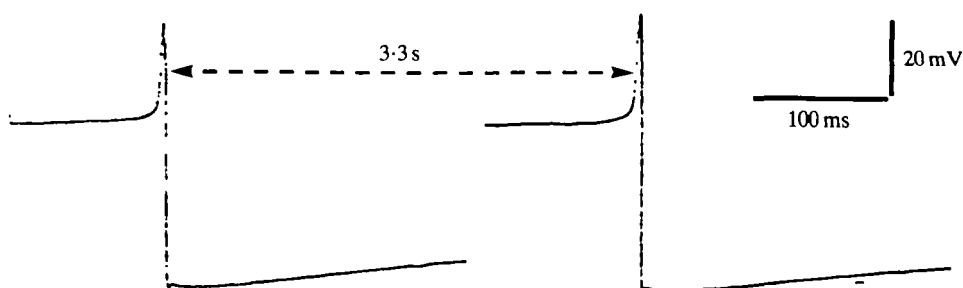


Fig. 14. Effect of 4-aminopyridine (4-AP). At a concentration of  $1 \text{ mmol l}^{-1}$ , 4-AP has no obvious effects on the repolarizing phase of the spikes which can occur rhythmically.

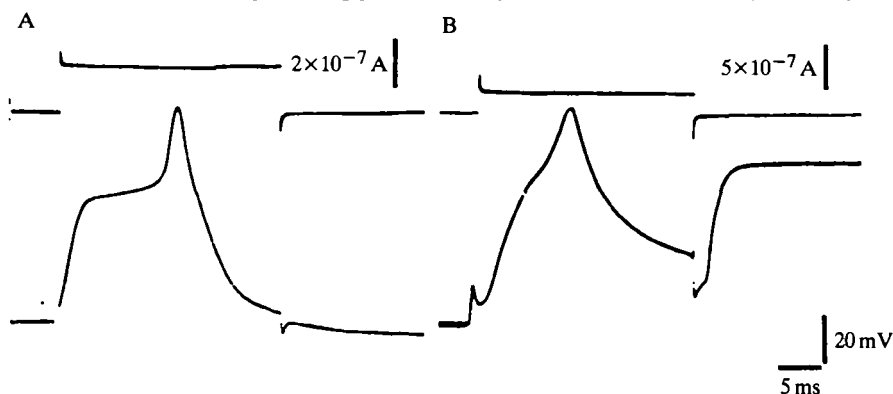


Fig. 15. Membrane response and contractile activity of the cells incubated in the presence of 4-aminopyridine (4-AP) ( $2 \text{ mmol l}^{-1}$ ) in  $\text{Na}^+$ -free solution containing (A) 50 and (B)  $100 \text{ mmol l}^{-1} \text{ Ca}^{2+}$  ions. Both figures show that action potentials are maintained, but contraction occurs only at the higher calcium concentration (B).

ciliates, such as *Paramecium* and *Stylonichia*, graded mechanical stimuli to the anterior region evoke graded depolarizing receptor potentials, which reverse ciliary beat (Naitoh & Eckert, 1969; de Peyer & Machemer, 1978). Unlike those of *Actinocoryne* however, these are  $\text{Ca}^{2+}$ -dependent (Naitoh, Eckert & Friedman, 1972; de Peyer & Machemer, 1977). Mechanical stimulation of the posterior region evokes hyperpolarizing receptor potentials carried by  $\text{K}^+$ , which accelerate forward ciliary beat (Naitoh & Eckert, 1973; de Peyer & Machemer, 1978), but in *Actinocoryne* as in *Stentor* (Wood, 1982) mechanical stimulation of different regions of the cell yields only depolarizing potentials.

In the sessile *Vorticella* it is not yet clear whether there are regenerative membrane responses (Shiono & Naitoh, 1978), whereas in *Zoothamnium* (Moreton & Amos, 1979) regenerative responses are found. In free-swimming ciliates, the responses to current injection may be nearly all-or-none (*Stylonichia*, some *Paramecium* mutants; Wood, 1982; Hinrichsen & Saimi, 1984) or, more usually, graded according to stimulus intensity (*Paramecium*, Naitoh & Eckert, 1968). Such graded responses can be experimentally converted into all-or-none action potentials by procedures which alter the  $\text{Ca}^{2+}$ -dependence or the inactivation of the  $\text{Ca}^{2+}$  current (Brehm & Eckert, 1978; Eckert & Brehm, 1979) rather than the  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  conductance (Brehm, Dunlap & Eckert, 1978).

In *Actinocoryne*, rapid all-or-none overshooting action potentials precede contraction in normal conditions. In experimental conditions, the action potentials may be carried by  $\text{Na}^+$  or by  $\text{Ca}^{2+}$ , but contraction requires the presence of external  $\text{Ca}^{2+}$ . In normal SW or ASW the following considerations suggest that whilst there is a small  $\text{Ca}^{2+}$  component, the action potential is predominantly carried by  $\text{Na}^+$ . (1) Action potentials in  $500 \text{ mmol l}^{-1} \text{ Na}^+$ ,  $\text{Ca}^{2+}$ -free solutions are similar to those in SW or ASW. (2) In  $\text{Na}^+$ -free solutions they are smaller and at  $10 \text{ mmol l}^{-1} \text{ Ca}^{2+}$  only evoked with difficulty by current injection. (3)  $\text{Co}^{2+}$  added to SW or ASW does not inhibit membrane electrogenesis, nor does the addition of  $1 \text{ mmol l}^{-1}$  EGTA to  $500 \text{ mmol l}^{-1} \text{ Na}^+/\text{Ca}^{2+}$ -free solutions, but in both cases action potentials evoked by current injection are not followed by contraction. (4) Rapid contractions following action potentials in  $\text{Na}^+$ -free solutions (see 2), and the absence of contractions following action potentials in solutions containing  $\text{Co}^{2+}$  or EGTA (see 3) suggest that in SW or ASW there is an entry of  $\text{Ca}^{2+}$  during the action potential.

Action potentials that are carried by  $\text{Na}^+$  and  $\text{Ca}^{2+}$  are, however, known from a variety of excitable tissues (Hagiwara & Byerly, 1981). These are all from metazoans; *Actinocoryne* is thus the first exception to Hille's (1984) generalization that sodium-requiring action potentials are unknown outside the metazoan animals, and it is the only protozoan studied which exhibits a mixed  $\text{Na}^+/\text{Ca}^{2+}$  action potential. Although in most ciliates there are  $\text{Ca}^{2+}$  spikes (Naitoh *et al.* 1972; Wood, 1982), a small  $\text{Na}^+$  conductance is known in some *Paramecium* mutants (Saimi & Kung, 1980; Hinrichsen & Saimi, 1984), but this is  $\text{Ca}^{2+}$ -activated, and hence abolished in  $\text{Ca}^{2+}$ -free solutions.

Although voltage clamp studies would be necessary to establish the different conductances during the action potential, the effects of TEA and 4-AP on the

repolarizing phase suggest that it is brought about by a voltage-activated  $K^+$  conductance. The increased duration of the action potential seen when  $Ca^{2+}$  entry is blocked by  $Co^{2+}$  and the plateau in  $10\text{ mmol l}^{-1}$   $Ca^{2+}/Na^+$ -free solution which disappears when  $Ca^{2+}$  is increased, suggest that a  $Ca^{2+}$ -activated  $K^+$  conductance may also be involved in repolarization.

In normal SW or ASW, all-or-none rapid contractions always follow action potentials with a delay of some 10 ms and cannot be evoked without a preceding action potential. It therefore seems probable that under normal conditions, contraction is triggered by the action potential. In other contractile protozoa, according to Naitoh (1982), contraction is not clearly linked to membrane electrical activity.

Our results show that external  $Ca^{2+}$  is required for rapid all-or-none contraction, but it is not clear how  $Ca^{2+}$  entry is related to contraction. Contraction evidently requires disassembly of axonemal microtubules, since if they are stabilized with  $D_2O$ , contraction is inhibited. In contracted specimens, the axonemal microtubules are disassembled and appear in random arrays in the base (Febvre-Chevalier, 1980).

The regulatory role of  $Ca^{2+}$  in microtubule assembly and disassembly is now clear (Schliwa, 1976), and it is known that an increase in internal  $Ca^{2+}$  induces disassembly (see Dustin, 1984). It is thus reasonable to suppose that the microtubular scaffolding of *Actinocoryne* is disassembled by the transient  $Ca^{2+}$  entry during the action potential.

However, normal contractions are so rapid that they can hardly be brought about by a microtubule sliding mechanism, and it is possible that the microfibrils seen in bundles in the base of contracted specimens may be involved. Whatever the mechanism of contraction may be, entry of external  $Ca^{2+}$  is required to trigger it, although evidently our observations do not exclude a subsequent linked  $Ca^{2+}$  release from intracellular stores, triggered by  $Ca^{2+}$  entry during the action potential. There are numerous small vesicles and dense granules within the cytoplasm which might act as such stores.

Under certain experimental conditions, instead of the rapid all-or-none contractions seen in SW or ASW, slow or incomplete contractions may follow mechanical or electrical stimulation. Thus, for example, normal rapid contractions are seen in  $Ca^{2+}$ -free solutions at first, but after prolonged sojourn contractions slow and may finally be abolished. Again, in solutions containing  $1\text{ mmol l}^{-1}$  EGTA, rapid contractions are blocked, but slow contractions may still take place. Since the mechanism of excitation-contraction coupling appears to be  $Ca^{2+}$ -dependent, such slow contractions persisting after EGTA or  $Co^{2+}$  treatment may perhaps be attributed to  $Ca^{2+}$  ions linked to the outer surface of the cell membrane, which is covered with a mucous coat. The axopods are not covered with a mucous coat and it is noteworthy that they do not contract under conditions where the stalk shows slow contractions.

We are unable to offer an explanation for the striking effect of 4-AP on contraction. In *Actinocoryne*, 4-AP blocks contraction in concentrations at which no obvious effects are exerted on the repolarizing phase of the action potential, i.e. at

concentrations below which its previously known action as a specific blocker of voltage-dependent  $K^+$  conductance (Pelhate & Pichon, 1974) is shown. Regenerative responses are obtained in  $Na^+$ -free solutions containing 4-AP and  $Ca^{2+}$ , so that it does not seem that 4-AP abolishes contraction by blocking  $Ca^{2+}$  entry.

Cell membranes are permeable to 4-AP (Bowman, 1982) so that it presumably acts internally, inhibiting one or more of the intracellular steps involved in contraction; that contraction takes place in the presence of 4-AP in  $Na^+$ -free solutions containing high levels of  $Ca^{2+}$  may suggest that there is a competitive interaction between 4-AP and internal  $Ca^{2+}$ -sensitive sites.

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