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CELLULAR CONTRACTION PRECEDES MEMBRANE DEPOLARIZATION IN *VORTICELLA CONVALLARIA*

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Summary

Application of a mechanical stimulus to the cell body of the peritrich ciliate *Vorticella convallaria* evoked an all-or-nothing membrane depolarization, the large pulse. This was always accompanied by an all-or-nothing cellular contraction, and simultaneous recordings of the two events revealed that the large pulse was always preceded by the cellular contraction. A smaller graded membrane depolarization (the medium pulse) was sometimes produced in response to a weaker mechanical stimulus. The medium pulse was accompanied by a small, graded, localized contraction of the cell body and was occasionally followed by a large pulse. When a large pulse occurred during a medium pulse, it reached the same peak level as that of a large pulse evoked without a preceding medium pulse. When a medium pulse occurred during a medium pulse, summation of the two pulses was observed. Sustained contraction causes *V. convallaria* to become rounded, and in this state a mechanical stimulus stronger than that used to evoke the large pulse evoked a graded depolarizing mechanoreceptor potential in the cell. We conclude that both the large and medium pulses are caused by an inward receptor current that is activated mechanically following contraction of the cell body. A localized contraction evokes a small mechanoreceptor current, causing a medium pulse. An all-or-nothing contraction evokes a saturated, all-or-nothing mechanoreceptor current, causing a large pulse.

Key words: *Vorticella convallaria*, membrane depolarization, cellular contraction, mechanoreceptor potential, Ca^{2+} requirement.

Introduction

A sessile peritrich ciliate, *Vorticella convallaria*, exhibits an all-or-nothing type of cellular contraction that involves shrinkage of the cell body and coiling of the stalk either spontaneously or in response to stimulation. The contraction is assumed to be a behavioural response effective in protecting the cell against dangerous mechanical disturbance such as turbulence in the surrounding water.

Moreton and Amos (1979) recorded two kinds of membrane depolarizing responses from the cell body of the peritrich ciliate *Zoothamnium geniculatum*, i.e. a large action-potential-like response associated with contraction of the stalk and a small depolarizing response associated with systole of the contractile vacuole. They suggested that the stalk contraction is triggered by the large membrane potential response in the same way as skeletal muscle contraction is triggered by an action potential. Similar observations were made in *V. convallaria* by Shiono *et al.* (1980), who described an action-potential-like large pulse associated with cellular contraction and a small pulse associated with systole of the contractile vacuole. They also suggested that the cellular contraction is triggered by the large pulse.

To investigate the causal relationship between the large pulse and cellular contraction in *V. convallaria*, we examined the timing of these two events. We also examined some electrophysiological characteristics of the cell in relation to the large pulse and the cellular contraction. In this paper, we also describe a graded membrane depolarizing response, which we call the medium pulse and which has an amplitude smaller than that of the large pulse but larger than that of the small pulse. The medium pulse was associated with a small, graded, localized cellular contraction. Some of the results described in this paper have been presented verbally elsewhere (Shiono and Naitoh, 1977, 1978).

Materials and methods

Cells of *Vorticella convallaria* (Shiono *et al.* 1980) were grown at 20 °C in a bacterized (*Bacillus pneumoniae*) wheat straw infusion. Conventional electrophysiological techniques for ciliates were employed (Naitoh and Eckert, 1972). Several cells detached from debris were pipetted into a thin hanging drop under a coverslip, which was mounted on an experimental

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vessel constructed on a microscope stage. A microneedle was inserted into the aboral region of the cell body to keep the cell in the visual field of the microscope. The standard saline solution containing (final concentration in mmol l^{-1}) 1.0 KCl, 1.0 $CaCl_2$ and 1.0 Tris–HCl buffer (pH 7.4) was then introduced into the vessel to bathe the impaled cell. Two glass microcapillary electrodes, one filled with 0.3 mol l^{-1} KCl (tip resistance 2×10^8 to $9\times10^8\,\Omega$) for membrane potential measurement and the other filled with 1 mol l^{-1} KCl (tip resistance 0.8×10^8 to $3\times10^8\,\Omega$) for current injection, were inserted into the cell body.

A mechanical stimulus was given to the cell body by hitting the cell surface with the tip of a glass microneedle (5 μm tip diameter) using a piezoelectric phonocartridge. The voltage applied to the cartridge is a measure of the relative strength of the stimulus.

Contraction of the impaled cell was monitored, using a phototransistor placed at the focal plane of the eyepiece of the microscope, by measuring the change in the amount of light in the visual field of the microscope resulting from the contraction. The output current from the phototransistor was amplified with a d.c. amplifier to monitor the degree and the time course of the contraction or with an a.c. amplifier to monitor just the occurrence of the contraction.

The membrane potential, the stimulation current, the electrical pulse for driving the microneedle and the electrical signal corresponding to cellular contraction were displayed on the screen of a cathode ray tube and photographed for later examination. All the experiments were performed at room temperature, which ranged from 20 to 25 °C.

Results

Spontaneous membrane potential responses

Sessile cells of *V. convallaria* showed a more or less stable resting membrane potential in standard saline solution (−44.1±2.6 mV; mean ± S.D., *N*=16). They occasionally exhibited three types of transient membrane depolarizing response, which differed in their amplitude and time course (Fig. 1).

The large response was all-or-nothing with a definite overshoot and was always associated with an all-or-nothing cellular contraction. The peak level was 29.8±11.2 mV, the amplitude 74.0±16.0 mV and the half-width was 453±166 ms (*N*=16). The large response corresponds to the ‘large pulse’ reported by Shiono *et al.* (1980).

The medium-sized response showed a wider variation in its amplitude and time course than the large pulse. The amplitude varied from 18.8 to 64.4 mV (*N*=8) and the half-width varied between 406 and 724 ms (*N*=8). The medium-sized response was occasionally associated with a minor cellular movement, such as a slight tilting of the cell body against the stalk and/or a small localized contraction of the cell body, especially in the aboral region. The amplitude of the response was larger when the cellular movement was more conspicuous. The medium-sized response will be termed the ‘medium pulse’.

The smallest response was always accompanied by pulsation of the contractile vacuole. The amplitude was 3.8±1.3 mV (*N*=3) and the half-width was 585±135 ms (*N*=3). The response corresponds to the ‘small pulse’ reported by Shiono *et al.* (1980).

Mechanical stimulus-evoked large pulse

A mechanical stimulus applied to the cell body evoked an all-or-nothing cellular contraction which was always accompanied by an all-or-nothing membrane depolarizing response with a definite overshoot. Representative responses of a single cell to mechanical stimuli of varying strengths are shown in Fig. 2.

A comparison between the mechanical stimulus-evoked depolarizing response and a spontaneously occurring large pulse in a single cell (Fig. 3) showed no obvious differences in the amplitude and the time course between the two. Hereafter, the mechanical stimulus-evoked depolarizing response will also be termed the ‘large pulse’.

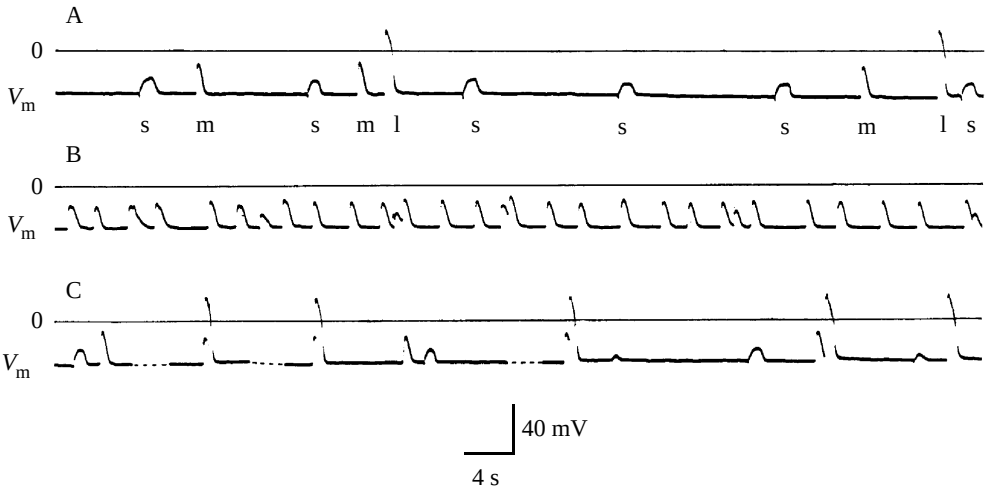

Fig. 1. Spontaneous membrane potential (V_m) responses exhibited by a single cell of *Vorticella convallaria*. (A) Large pulses (l), medium pulses (m) and small pulses (s). (B) A train of medium pulses. (C) Some large pulses superimposed on the medium pulse. Lines labelled 0 are the reference level for V_m.

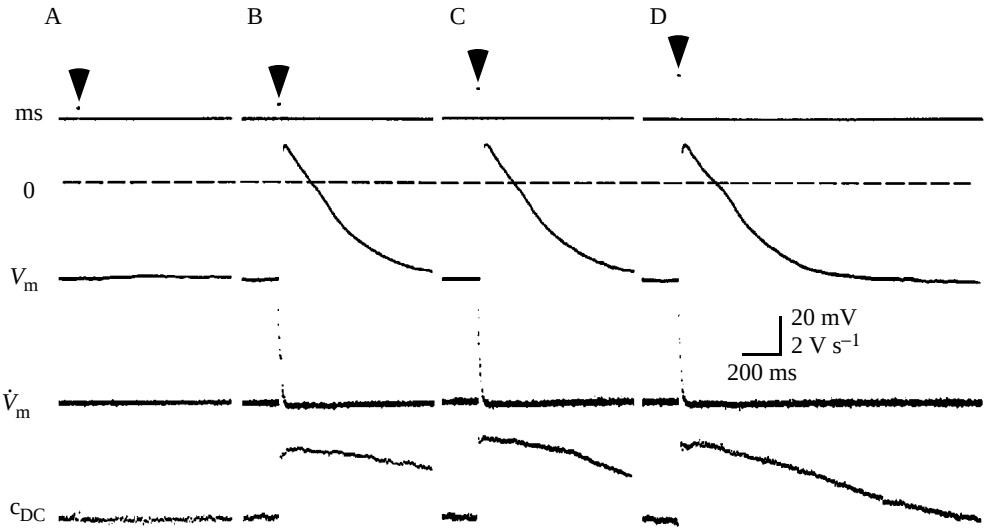

Fig. 2. Transient membrane depolarizations (the large pulses) and all-or-nothing cellular contractions evoked by mechanical stimuli of different strengths obtained from a single cell of *V. convallaria*. (A) The stimulus strength (arrowheads) was too weak to evoke any response. (B) The stimulus strength was just enough to evoke a response. (C,D) The stimulus strength was stronger than that in B (C<D). V_m, membrane potential; $\dot{V}_m$, first-order time derivative of V_m; c_{DC}, electrical signals corresponding to the cellular contraction amplified by a d.c. amplifier (relative degree of cellular contraction); ms, square current pulses (arrowheads) applied to a piezoelectric phonocartridge to drive a microneedle against the cell body surface to stimulate the cell mechanically (relative stimulus strength). The dotted line labelled 0 is the reference level for V_m.

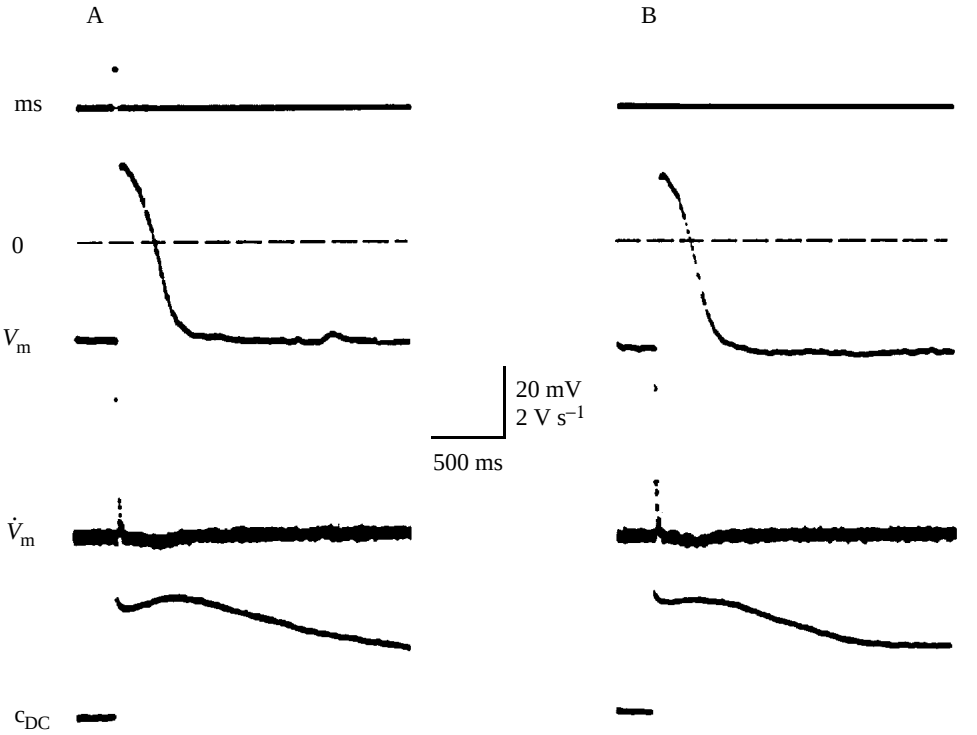

Fig. 3. A mechanical stimulus-evoked transient membrane depolarization (A) and a spontaneous large pulse (B) obtained from a single cell of *V. convallaria*. V_m, membrane potential; $\dot{V}_m$, first-order time derivative of V_m; c_{DC}, electrical signals corresponding to the cellular contraction amplified by a d.c. amplifier (relative degree of cellular contraction); ms, square current pulses applied to a piezoelectric phonocartridge to drive a microneedle against the cell body surface to stimulate the cell mechanically. The dotted line labelled 0 is the reference level for V_m.

Phase-plane trajectory of the large pulse

The time course of the rising phase of a large pulse (Fig. 4B) was examined on its phase-plane trajectory (Jennerick, 1963). In Fig. 4A, the first-order time derivative of the membrane potential is plotted against the corresponding membrane potential. The plot was linear for the rising phase of the large pulse except for its very early portion. The straight trajectory implies that the membrane potential changes exponentially with time. The time constant determined from the slope of the straight portion of the trajectory is 8.1±1.1 ms (N=5) (7.9 ms for the trace in Fig. 4A).

Time difference between the onset of the large pulse and that of cellular contraction

A large pulse and the electrical signal that accompanies cellular contraction were simultaneously displayed on the screen of a cathode ray tube at a high sweep velocity, and the time difference between the onset of the two electric signals

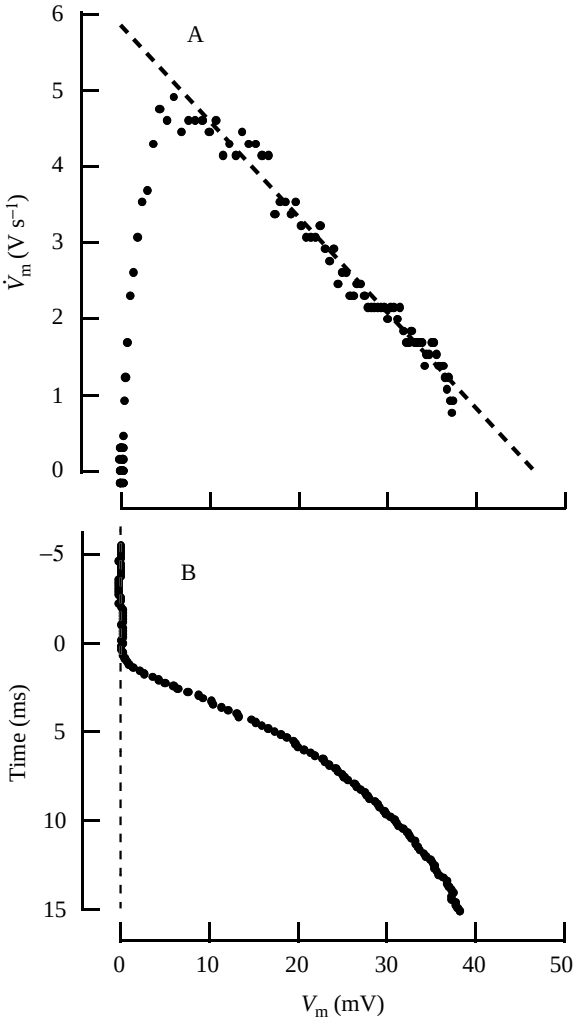

Fig. 4. Phase-plane trajectory of the rising phase of a large pulse evoked by mechanical stimulation (A) and the corresponding rising phase of the large pulse (B) obtained in a single cell of *V. convallaria*. V_m, the membrane potential with reference to the resting membrane potential; $\dot{V}_m$, the first-order time derivative of V_m. The broken line in A is a linear regression line (r^2=0.999). Time 0 in B corresponds to the time when a mechanical stimulus was applied to the cell body.

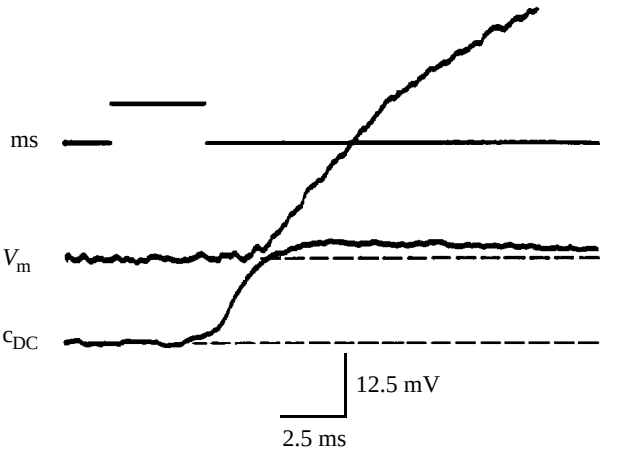

Fig. 5. Simultaneous recordings of a mechanical stimulus-evoked large pulse and the cellular contraction obtained from a single cell of *V. convallaria*. V_m, the membrane potential with reference to the resting membrane potential; c_{DC}, electrical signals corresponding to the cellular contraction amplified by a d.c. amplifier (relative degree of cellular contraction); ms, a square electrical pulse applied to a piezoelectric phonocartridge to drive a microneedle against the cell body.

was measured. As shown in Fig. 5, the contraction preceded the large pulse. The time difference was 2.3 ms (1.8±0.7 ms; N=3).

The input resistance and the input capacitance of the cell during the large pulse

The change in the input resistance during the large pulse was determined by injecting a train of square current pulses into the cell body while the large pulse was evoked by a mechanical stimulus. As shown in Fig. 6B, an exponentially decaying potential change caused by each current pulse was observed superimposed on the large pulse. The input resistance corresponding to each pulse was estimated from each steady potential shift at the end of each pulse and plotted against the time after the onset of the mechanical stimulus (Fig. 7). The input resistance was lower during the earlier portion of the large pulse and increased exponentially with time to resume its original resting value as the large pulse subsided. Extrapolation of the plot to time 0 gives the lowest value of 23.5±2.5 % (N=8) (approximately 22 % in Fig. 7) of the resting input resistance of the cell. In contrast to these results, the input resistance of the cell did not change during a simulated large pulse obtained by injecting a triangular inward current into the cell body (Fig. 6D,E). The simulated large pulse was not accompanied by a cellular contraction.

The peak value of the first-order time derivative of each membrane potential response to each injected current pulse was unchanged during the large pulse (Fig. 6B,C), implying that the input capacitance of the cell does not change during the large pulse.

The refractoriness of the large pulse and the cellular contraction in response to mechanical stimulation

The refractoriness of the large pulse and that of the cellular contraction in response to mechanical stimulation were examined by comparing two responses evoked by two successive mechanical stimuli applied at different intervals. A representative series of results is shown in Fig. 8. The large pulse evoked by the second stimulus was identical with that evoked by the first stimulus when the stimulus interval was 12 s (Fig. 8A,B). The second large pulse decreased only slightly in amplitude when the stimulus interval was reduced to 800 ms (Fig. 8C–E), but decreased markedly when the interval was less than 400 ms and disappeared at an interval of less than approximately 200 ms (Fig. 8G–I).

The second contraction was identical with the first when the stimulus interval was more than 12 s (Fig. 8A,B) but was

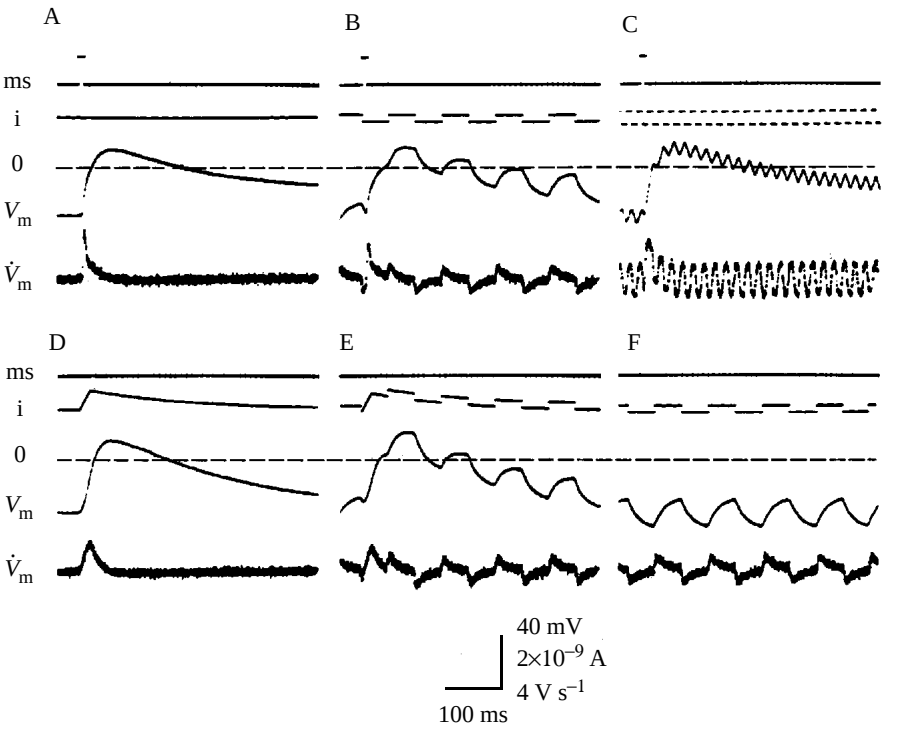

Fig. 6. Membrane potential responses to a train of square current pulses injected into the cell body during a large pulse evoked by a mechanical stimulus (A–C) and during a membrane depolarization evoked by injection of a triangular current pulse (D,E) obtained from a single cell of *V. convallaria*. (A,D) No current pulses were injected. (B,E,F) The frequency of the current pulses was 10 Hz. (C) The frequency of the current pulses was 50 Hz. (F) Effect of current injection in the absence of a large pulse. V_m, membrane potential; $\dot{V}_m$, first-order time derivative of V_m; ms, square current pulses applied to a piezoelectric phonocartridge to drive a microneedle against the cell body surface to stimulate the cell mechanically (relative stimulus strength); i, current injected into the cell body. The dotted line labelled 0 is the reference level for V_m.

always slightly larger than the first when the stimulus interval was shorter than 4 s (Fig. 8C–F). A slightly larger second contraction was still observed when the stimulus interval was less than 200 ms (Fig. 8G,H), even though the second large pulse was not observed at this interval. When the stimulus interval was less than 50 ms, only a single contraction was observed (Fig. 8I).

Examination of two successive spontaneous pulses

To examine the relationship between the large pulse and the medium pulse, the time interval between the peaks of two successive spontaneous pulses was determined in a train of 161 large and medium pulses exhibited by a single cell in 76 min. All pairs of successive pulses were categorized into four groups according to the sequence of their appearance: (1) medium pulse to large pulse (M–L), (2) medium pulse to medium pulse (M–M), (3) large pulse to medium pulse (L–M), and (4) large pulse to large pulse (L–L). A frequency distribution was determined for each group (Fig. 9). The modal interval for the M–L pairs (Fig. 9A) was less than 0.5 s, while that for the L–M pairs (Fig. 9B) and for the L–L pairs (Fig. 9C) was 32–64 s. The modal interval for the M–M pairs was 8–16 s. The mean interval for all the pairs was approximately 31 s.

When a large pulse occurred during a preceding medium pulse (Fig. 10B,C, see also Fig. 1C), there appeared to be no summation of the two pulses, with the peak level of the large pulse being approximately the same as that of a large pulse occurring independently of a medium pulse (Fig. 10A). In contrast, when a medium pulse occurred during a preceding medium pulse, summation of the two pulses was observed (see Fig. 1B).

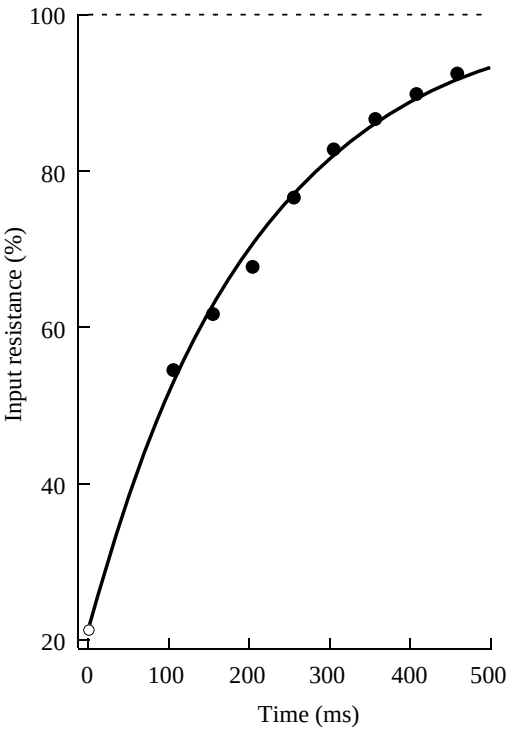

Fig. 7. Time course of changes in the input resistance of a cell of *V. convallaria* during a mechanical stimulus-evoked large pulse. The input resistance is expressed as a percentage of its resting value. Each input resistance measured at the end of each current pulse was plotted against the time from the start of the mechanical stimulus. The solid line is the exponential curve drawn according to the least-squares method (χ^2=15.86). The input resistance at 0 time (open circle) was obtained by extrapolation of this line.

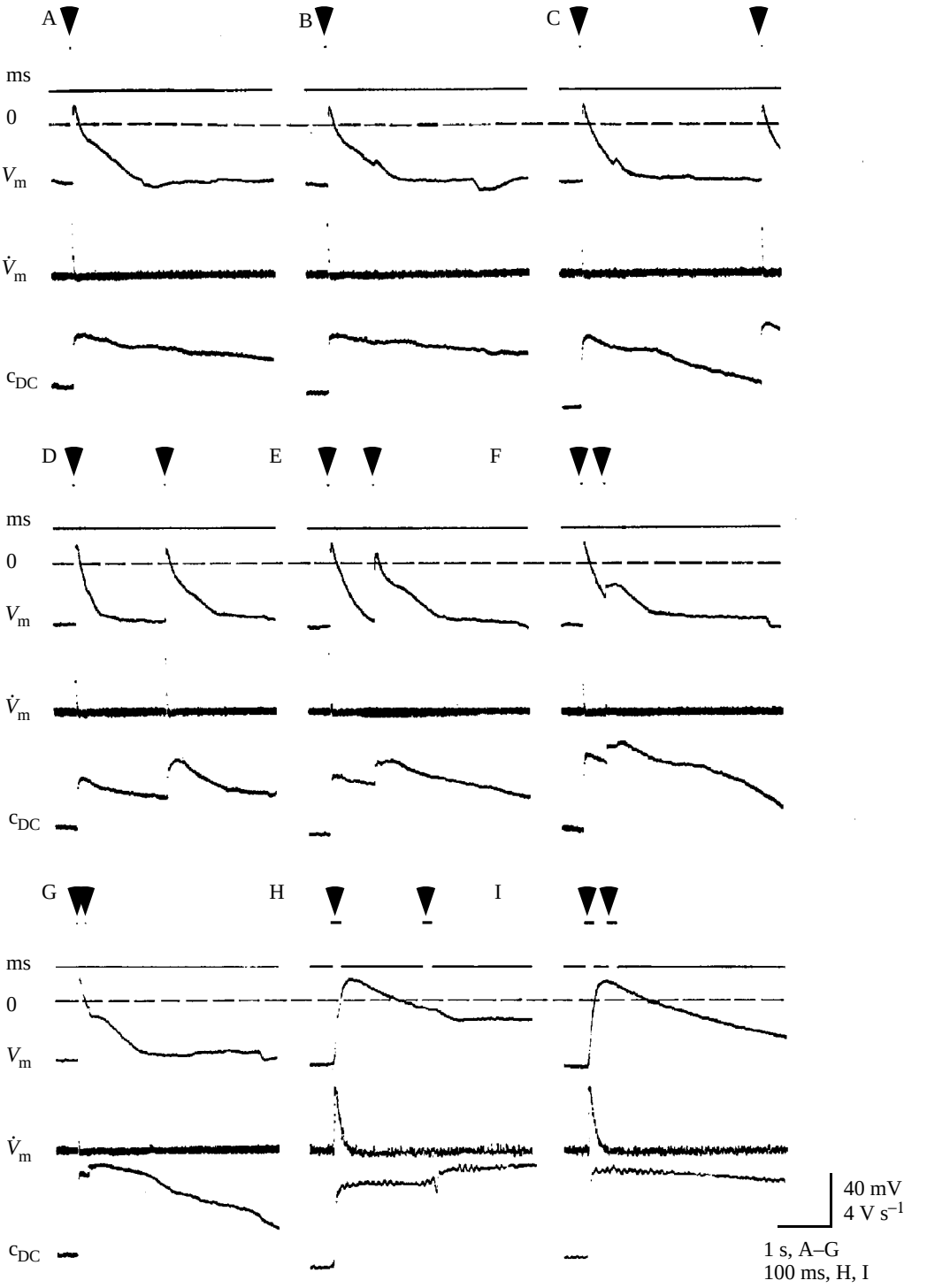

Fig. 8. The large pulses and the cellular contractions evoked by two successive mechanical stimuli applied to the cell body of a single cell of *V. convallaria* at various intervals. (A,B) A pair of responses at an interval of 12 s. (C–I) Two successive responses at various intervals. In H and I, the sweep velocity of the cathode ray tube was increased 10-fold. In G and H, the same response is displayed at different sweep velocities. V_m, membrane potential; $\dot{V}_m$, first-order time derivative of V_m; c_{DC}, electrical signals corresponding to the cellular contraction amplified by a d.c. amplifier (relative degree of cellular contraction); ms, square current pulses (arrows) applied to a piezoelectric phonocartridge to drive a microneedle against the cell body surface to stimulate the cell mechanically. The dotted line labelled 0 is the reference level for V_m.

Mechanical stimulus-evoked medium pulse

A mechanical stimulus applied to the aboral region of the cell body sometimes evoked a depolarizing response similar to the spontaneous medium pulse in its amplitude, rate of rise and half-width and in the absence of an all-or-nothing cellular contraction (Fig. 11B). The stimulus-evoked medium pulse was sometimes followed by a large pulse accompanied by an all-or-nothing contraction, especially when the stimulus intensity was slightly higher than that required to evoke only a medium pulse (Fig. 11C). As in the case of spontaneously occurring medium and large pulses (Fig. 10B,C), there was no summation between the two pulses. A large pulse without a preceding medium pulse was evoked when the stimulus intensity was further increased, as mentioned previously (Fig. 11D). The medium pulse was occasionally accompanied by localized cellular movement such as contraction of the aboral region of the cell body and tilting of the cell body against the stalk, responses similar to those observed after a spontaneous medium pulse.

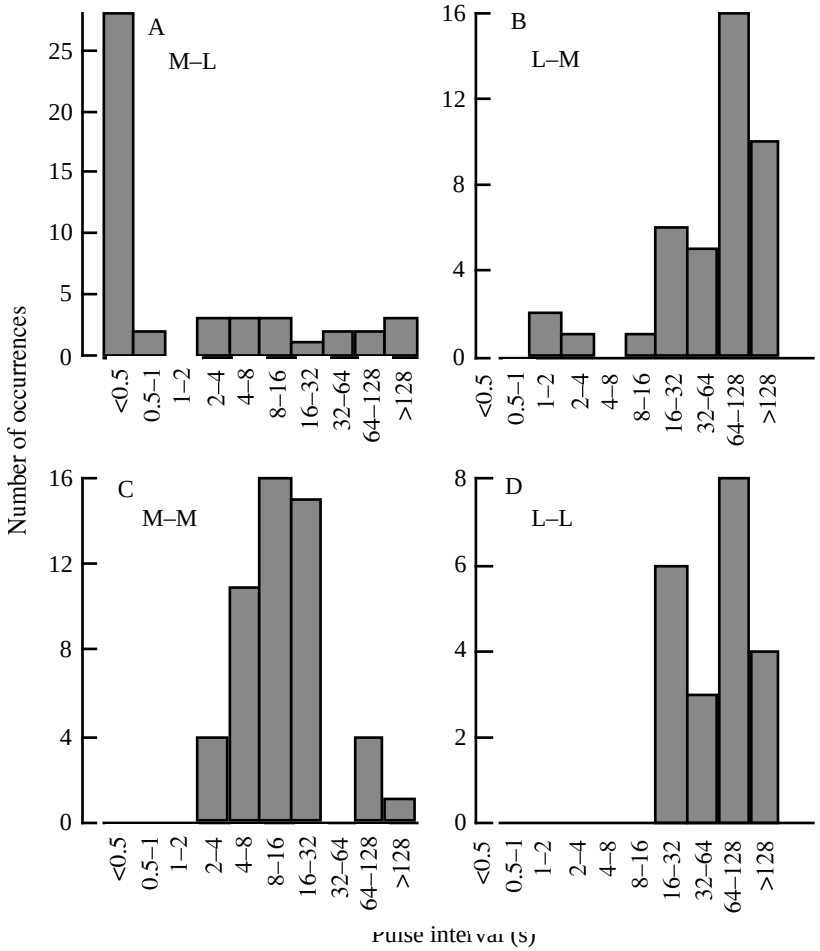

Fig. 9. Frequency distribution of the interval between two successive pulses examined in a train of pulses (the large, L, and medium, M, pulses) exhibited by a single cell of *V. convallaria*. See the text for details.

Depolarizing mechanoreceptor potential

V. convallaria showed sustained contraction of the cell body in standard saline solution after repeated exposure to a K^+-rich (32mmol l^{-1}) solution and became rounded. Rounded cells produced a transient membrane depolarization in response to a mechanical stimulus 10–25 times stronger than the mechanical stimulus just strong enough to evoke a large pulse in a normal bell-shaped cell. The amplitude and the maximum rate of rise of the response were larger when the stimulus intensity was higher (Fig. 12).

Some electrophysiological properties of the large pulse

Effects of the concentrations of the external ions

The membrane of *V. convallaria* was depolarized by an increase in external K^+ or Ca^{2+} concentration. Representative examples of the effects of the concentrations of these two

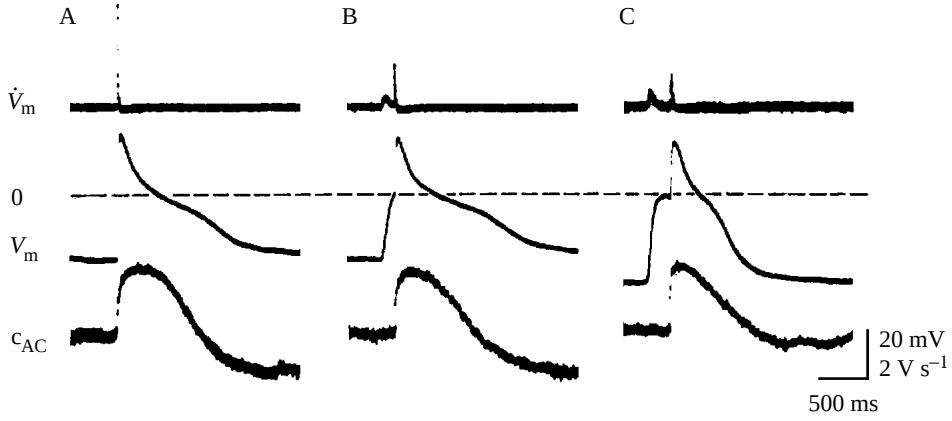

Fig. 10. Spontaneous large and medium pulses recorded from a single cell of *V. convallaria*. (A) A single large pulse. (B,C) A large pulse occurring during a medium pulse. V_m, membrane potential; $\dot{V}_m$, first-order time derivative of V_m; c_{AC}, electrical signals corresponding to the cellular contraction amplified by an a.c. amplifier (the trace shows only occurrence of the contraction). The dotted line labelled 0 is the reference level for V_m.

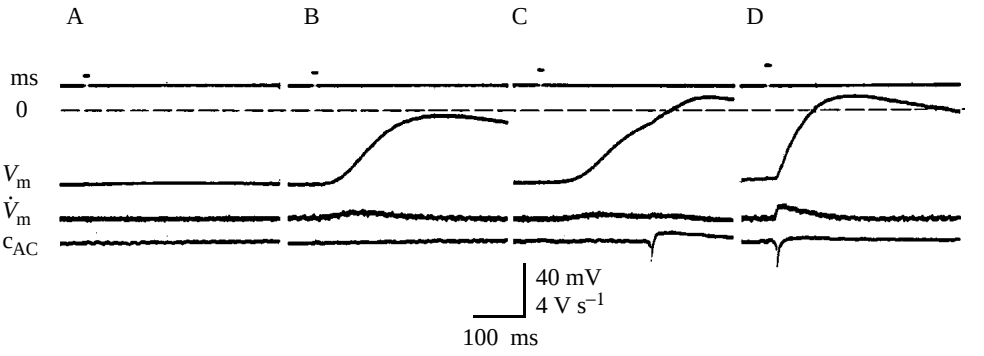

Fig. 11. Mechanical stimulus-induced medium and large pulses recorded from a single cell of *V. convallaria*. Stimulus strength was increased from A to D. (A) No response was evoked. (B) A medium pulse was evoked. (C) A medium pulse was followed by a large pulse, which was accompanied by a contraction (c_{AC}). (D) A large pulse was evoked without a detectable medium pulse. V_m, membrane potential; $\dot{V}_m$, first-order time derivative of V_m; c_{AC}, electrical signals corresponding to the cellular contraction amplified by an a.c. amplifier (the trace shows only the occurrence of the contraction); ms, square current pulses applied to a piezoelectric phonocartridge to drive a microneedle against the cell body surface to stimulate the cell mechanically (relative stimulus strength). The dotted line labelled 0 is the reference level for V_m.

cations on the resting membrane potential and on the peak of the large pulse are shown in Fig. 13. When $[K^+]$ was increased over a range from 4 to 32 mmol l^{-1}, a depolarization of 42±8.7 mV (N=5) (approximately 52 mV for the plot in Fig. 13A) was observed per 10-fold increase in $[K^+]$. When $[Ca^{2+}]$ was increased over a range from 0.1 to 10 mmol l^{-1}, a 19.4±3.4 mV (N=5) (approximately 22 mV for the plot Fig. 13B) depolarization was seen per 10-fold change in $[Ca^{2+}]$. The peak level of the large pulse was not affected by $[K^+]$, while it shifted in the depolarizing direction in increased external $[Ca^{2+}]$ at a rate of 19.3±7.1 mV (N=5) per 10-fold increase in $[Ca^{2+}]$ over the range from 0.01 to 10 mmol l^{-1} (approximately 21 mV for the plot in Fig. 13B). A decrease in the external Cl^- concentration achieved by substituting propionate for Cl^-did not affect the resting membrane potential level or the peak of the large pulse (results not shown).

The reversal potential for the large pulse

A mechanical stimulus was applied to evoke a large pulse while the membrane potential level was shifted by injecting current into the cell body. A representative set of results obtained from a single cell is shown in Fig. 14. The amplitude of the large pulse increased as the membrane was hyperpolarized (Fig. 14A) and decreased as the membrane was depolarized. The large pulse changed its polarity when the membrane potential was more positive than a certain value, the reversal potential (Fig. 14E). To estimate the reversal potential, the amplitude of the large pulse was plotted against the corresponding membrane potential (Fig. 15). The potential corresponding to the intersection between the best-fitting line drawn and a line corresponding to 0 amplitude (x-axis) is regarded as the reversal potential (32.8±5.0 mV; N=6; 32.2 mV for the plot in Fig. 15).

Responses to electrical current injection

The membrane potential changed exponentially with time when a square current pulse (duration 500 ms) smaller than 5×10^{-10} A was injected into the cell body (Fig. 16A,F). The input resistance of the cell was estimated to be $1.4\times10^{8}\pm0.1\times10^{8}\,\Omega$ (N=8) by dividing the steady membrane potential shift by the intensity of the current applied. The time constant for the exponential change was 33.0±4.6 ms (N=8).

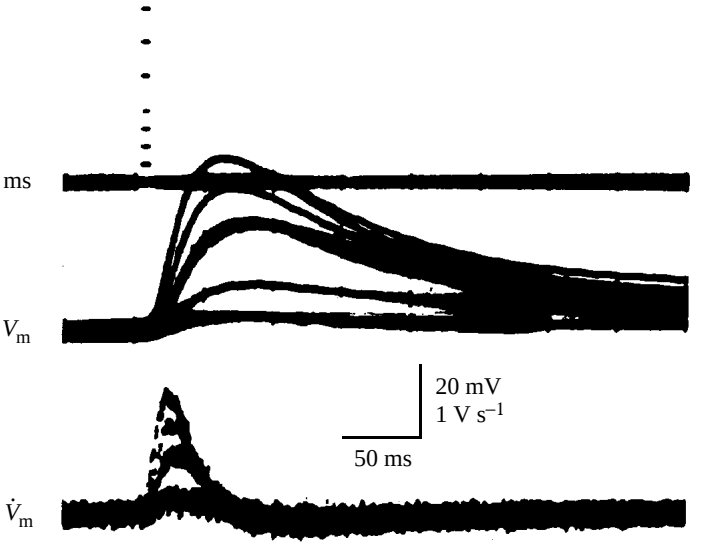

Fig. 12. Superimposed traces of the membrane potential responses to mechanical stimuli of varied strengths in a single cell of *V. convallaria* showing a sustained contraction. V_m, membrane potential; $\dot{V}_m$, first-order time derivative of V_m; ms, square current pulses applied to a piezoelectric phonocartridge to drive a microneedle against the cell body surface to stimulate the cell mechanically (relative stimulus strength). Some traces overlap and cannot be discriminated. The largest and the smallest responses correspond to the largest and the smallest mechanical stimuli, respectively.

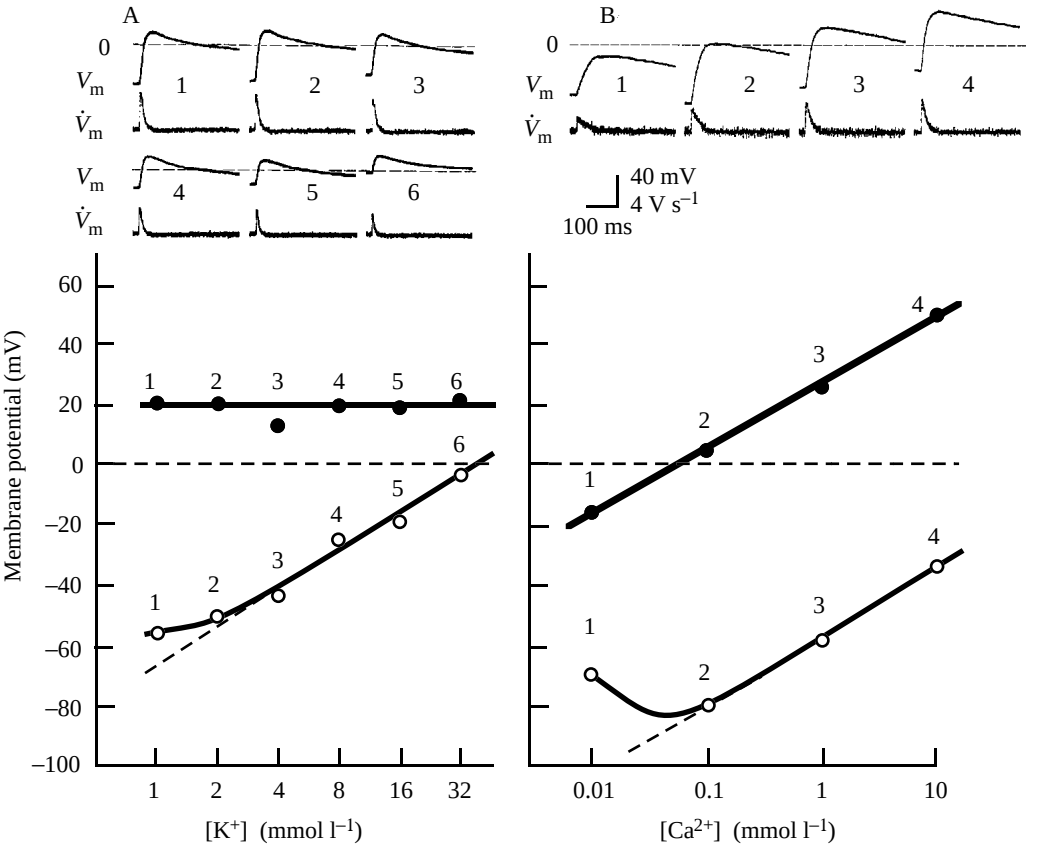

Fig. 13. Representative effects of K^+ concentration (A) and Ca^{2+} concentration (B) on the resting membrane potential (open circles) and the peak level of the large pulse (filled circles) obtained from a single cell of *V. convallaria*. Each set of traces in the upper portion of A and B shows the large pulses obtained from a cell bathed successively in a series of solutions with different K^+ or Ca^{2+} concentrations. The numbers beside the graph correspond to the numbered traces in the upper part of the figure. V_m, membrane potential; $\dot{V}_m$, first-order time derivative of V_m. Each dotted line labelled 0 is the reference level for V_m.

The input capacitance of the cell was estimated to be 230±32 pF (N=8) by dividing the time constant by the input resistance.

When the intensity of the outward current pulse was greater than 5×10^{-9} A and the exponential depolarizing membrane potential shift was greater than approximately 50 mV, the potential shift was followed by a more or less spike-like depolarizing response (Fig. 16C,D). In contrast to the large pulse, the outward current-evoked depolarizing spike was not accompanied by a cellular contraction (compare Fig. 16D with Fig. 16E).

When the intensity of the inward current pulse was greater than approximately 5×10^{-10} A, the exponential hyperpolarizing membrane potential shift was followed by a sudden shift of the membrane potential level towards the depolarizing direction. The membrane remained slightly depolarized for a while after the inward current pulse was turned off (Fig. 16G,H). The time from the onset of an inward current pulse to the start of the depolarizing response (the latent period for the response) was shorter when the current intensity was higher (Fig. 16G–J).

When the current intensity was higher than approximately 6.3×10^{-10} A, the large pulse was observed superimposed on the current-evoked membrane potential change and was accompanied by an all-or-nothing cellular contraction (Fig. 16I,J). The latent period for the large pulse was shorter when the current intensity was higher.

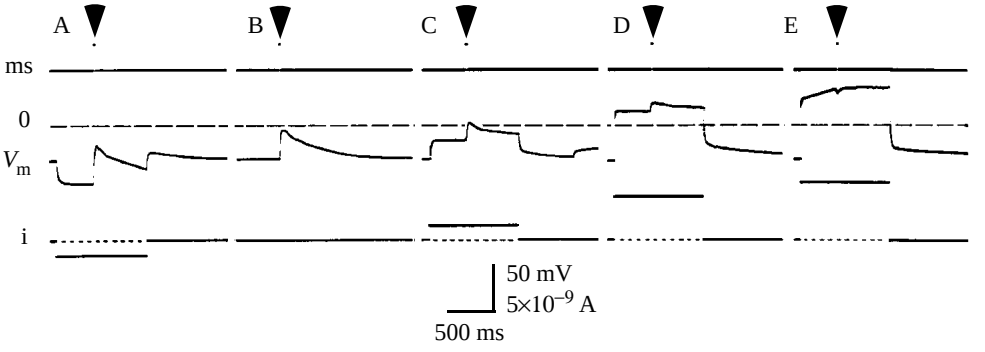

Fig. 14. Mechanical stimulus-evoked large pulses at various membrane potentials achieved by current injection obtained from a single cell of *V. convallaria*. V_m, membrane potential; ms, square current pulses (arrowheads) applied to a piezoelectric phonocartridge to drive a microneedle against the cell body surface to stimulate the cell mechanically (relative stimulus strength); i, current injected into the cell body. The dotted line labelled 0 is the reference level for V_m.

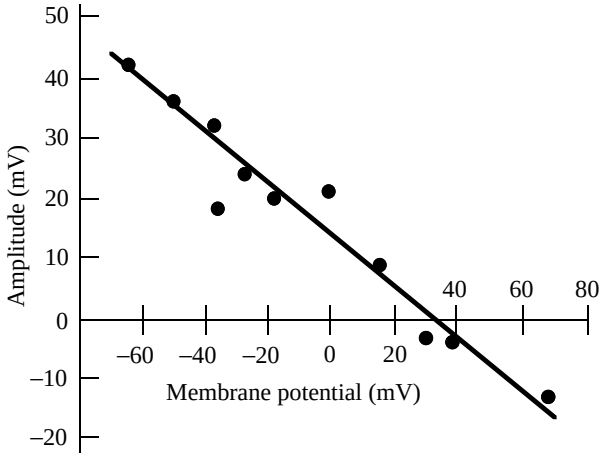

Fig. 15. A representative plot of the relationship between the amplitude of the mechanical stimulus-evoked large pulse (ordinate) and the membrane potential level (abscissa) obtained in a single cell of *V. convallaria*. Positive amplitude means that the large pulse is depolarizing, while negative amplitude means that the pulse is hyperpolarizing. The line is a linear regression line (r^2=0.999).

Ca^{2+} requirement for the cellular contraction

V. convallaria exhibited a large pulse and cellular contraction in response to a mechanical stimulus even in an EGTA-containing solution (1 mmol l^{-1} KCl + 2 mmol l^{-1} EGTA–Tris; pH 7.2) with a very low (less than 10^{-8} mol l^{-1}) Ca^{2+} concentration, although the amplitude of the pulse was small and unstable (data not shown). Non-impaled cells grown on a glass slide and immersed in EGTA-containing solution for more than 6 h contracted more than 200 times in response to a tap on the glass slide given at 20 s interval (data not shown). In contrast to mechanically evoked or spontaneously evoked contractions, an inward current pulse did not evoke cellular contraction in EGTA-containing solution (Fig. 17).

Discussion

Coupling between the large pulse and cellular contraction

Simultaneous recordings of the large pulse and the cellular contraction (Fig. 5) clearly demonstrated that contraction always preceded the large pulse. This implies that the large pulse is not the cause of the contraction, but is itself dependent on the contraction. In other words, the cellular contraction is not triggered by the large pulse, unlike the contraction of skeletal muscle fibres which is triggered by an action potential.

A membrane depolarization to a level comparable to the peak of the large pulse (approximately 30 mV) by outward current injection into the cell did not evoke a cellular contraction (Figs 6D, 16B), whereas a membrane hyperpolarization by inward current injection evoked a cellular contraction accompanied by a large pulse (Fig. 16I,J). These findings also support the idea that the large pulse does not trigger the cellular contraction. Thus, a previous statement by Shiono *et al.* (1980) that the large pulse triggers the cellular contraction in *V. convallaria* is no longer valid.

We have demonstrated that a graded membrane depolarization was evoked in response to mechanical stimulation in a cell of *V. convallaria* caused to undergo sustained contraction and to become rounded (Fig. 12). This indicates the presence of mechanosensitive ion channels in the cell membrane. Presumably, therefore, the cellular contraction induces a mechanical stress of the membrane, which activates

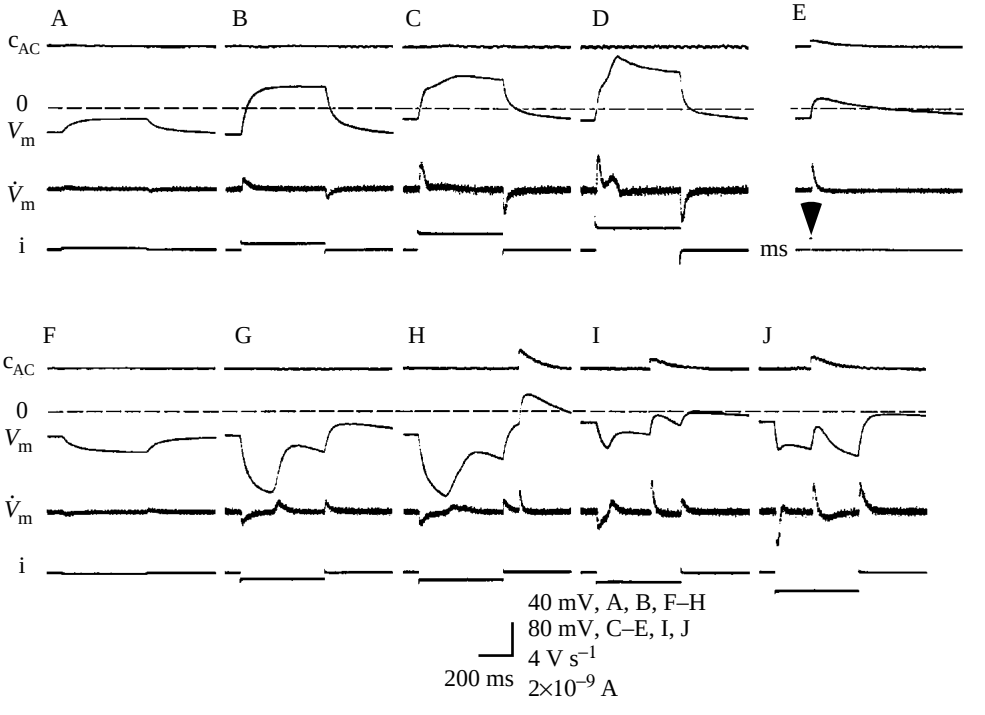

Fig. 16. Membrane potential changes and cellular contraction in response to injection of current pulses of various intensities into the cell body of *V. convallaria*. (A–D) The responses to outward current pulses (current intensity increases from A to D). (F–J) The responses to inward current pulses (current intensity increases from F to J). (E) A mechanical stimulus-evoked large pulse. V_m, membrane potential; $\dot{V}_m$, first-order time derivative of V_m; ms, square current pulse (arrowhead) applied to a piezoelectric phonocartridge to drive a microneedle against the cell body surface to stimulate the cell mechanically (relative stimulus strength); i, current injected into the cell body; c_{AC}, electrical signals corresponding to the cellular contraction amplified by an a.c. amplifier (the trace shows only the occurrence of the contraction). The dotted line labelled 0 is the reference level for V_m.

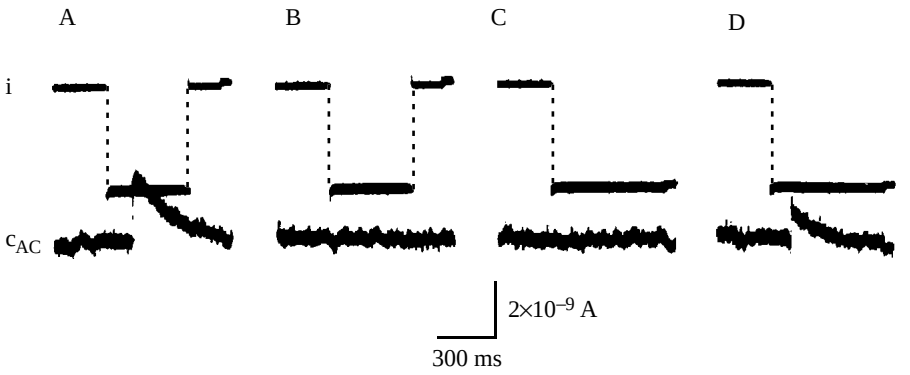

Fig. 17. Cellular contraction evoked by injection of an inward current pulse into the cell body of a single cell of *V. convallaria*. (A) In the presence of the external Ca^{2+} (1 mmol l^{-1}). (B,C) In the absence of the external Ca^{2+} (reduced to less than 10^{-8} mol l^{-1} by 2 mmol l^{-1} EGTA). The pulse in C was twice as long as that in B. (D) After reintroduction of Ca^{2+} into the external solution. c_{AC}, electrical signals corresponding to the cellular contraction amplified by an a.c. amplifier (the trace shows only the occurrence of the contraction); i, current injected into the cell body.

the mechanosensitive channels to generate a mechanoreceptor potential, corresponding to the large pulse. The slow decay of the large pulse is assumed to be caused by slow relaxation of the contraction-induced mechanical stress of the membrane. In a rounded cell, the mechanoreceptor channels are assumed to deactivate during the sustained contraction and to become ready for reactivation when stimulated mechanically. Activation of ion channels during the large pulse was demonstrated by the decrease in the input resistance of the cell to 23.5 % of its resting value (Figs 6B, 7).

The input resistance did not change during a simulated large pulse evoked by injection of a triangular current. The simulated large pulse was not accompanied by a cellular contraction (Fig. 6D). These results indicate that a membrane depolarization to the level of the large pulse does not activate depolarization-sensitive ion channels (see also Fig. 16B) and that depolarization-activated ion channels are not involved in the generation of the large pulse.

In this connection, it is interesting to note that in a carnivorous ciliate, *Didinium nasutum*, a depolarizing spike was always preceded by discharge of the extruding organelles (pexicysts and toxicysts; poisonous spears thrust against the prey, *Paramecium*). The spike was a mechanoreceptor potential generated by the activation of mechanosensitive channels in the proboscis membrane when the organelles were extruded through the proboscis (Hara *et al.* 1985).

We have demonstrated that the second cellular contraction was the same size as (or slightly larger than) the first even when the stimulus interval was as short as 200 ms (Fig. 8H). Two successive contractions fused when the stimulus interval was 50 ms (Fig. 8I). However, the large pulse in response to the second stimulus was always smaller than that in response to the first when the stimulus interval was shorter than 4 s (Fig. 8C–F). It is noticeable that relaxation of the cellular contraction of *V. convallaria* is slow as long as its half-decay time is longer than 4 s (the c_{DC} traces of Figs 2, 3, 5, 8) (Jones *et al.* 1970; Katoh and Naitoh, 1992). The second contraction, therefore, started while the cell was still in a more or less contracted state when the stimulus interval was less than 4 s. The second contraction-induced mechanical stress of the membrane is assumed to be smaller because the difference between the extent of contraction at the start and that at the peak of the second contraction is smaller. The second large pulse, therefore, became smaller at shorter stimulus intervals (Fig. 8C–F). The second contraction was not effective in evoking the large pulse at a stimulus interval of 200 ms, since the change in the extent of contraction was too small to induce a mechanical stress of the membrane strong enough to evoke the large pulse (Fig. 8G–I). The refractoriness of the large pulse observed in Fig. 8 is therefore ‘apparent’ and not ‘true’ refractoriness of the mechanoreceptor potential. Two successive mechanical stimuli to a rounded cell of *V. convallaria* are needed to examine the refractoriness of the mechanoreceptor potential.

Moreton and Amos (1979) reported in *Z. geniculatum* that coiling of the stalk evoked by mechanical stimulation of the cell body was observed approximately 10 ms after the start of a depolarizing action potential recorded from the cell body. This delay is comparable to the latent period for skeletal muscle contraction triggered by an action potential (Hodgkin and Horowicz, 1957). They therefore suggested that coiling of the stalk is triggered by the action potential. However, it is highly probable that the action potential of *Z. geniculatum* corresponds to the large pulse of *V. convallaria*, so how does such a delay occur? Assuming that mechanical stimulation of the cell body evokes the contraction first in the cell body and then in the stalk, after some delay, then if the delay is longer than the latent period for evoking a large pulse in the cell body (approximately 1.8 ms in *V. convallaria*; Fig. 5), the large pulse will precede the coiling of the stalk. The maximum delay due to conduction of the contraction from its initiation site on the cell body to the junction between the cell body and the stalk can be estimated as approximately 1 ms on the basis of the conduction velocity (approximately 2×10^{-1} m s^{-1}; Sugi, 1960) and the maximum conduction distance (approximately 2×10^{-4} m; from the oral edge to the junction). This value is too small to explain the 10 ms delay, and this may indicate that the delay occurs at the junction. Jones *et al.* (1970) reported that coiling of the stalk was evoked independently of contraction of the cell body. There are some structural differences, especially in their membrane-bound tubular and/or reticular systems, between the myonemes responsible for the contraction of the cell body and the spasmoneme responsible for the coiling of the stalk (Allen, 1973; Amos, 1972). Therefore, there might be a hypothetical process for

transmission of the contraction from the cell body to the stalk at the junction that is responsible for the delay. These membranous systems are now regarded as Ca^{2+} storage sites for activating the contractile mechanisms of vorticellid ciliates (Katoh and Kikuyama, 1997). Further examination of the cellular contraction in these vorticellid ciliates is needed to allow an explanation of the delayed coiling of the stalk.

The relationship between the medium pulse and the large pulse

The frequency distribution of the interval between two successive spontaneous pulses (Fig. 9) clearly demonstrated that the modal interval was shorter than 0.5 s in pulse pairs where a medium pulse was followed by a large pulse (M–L pairs), while it was 32–64 s in pulse pairs where a large pulse was followed by a medium pulse. In addition, the medium pulse could be evoked by a mechanical stimulus weaker than that required to evoke a large pulse (Fig. 11B). The medium pulse was followed by a large pulse when the stimulus intensity was slightly increased (Fig. 11C). A large pulse was evoked without a medium pulse by a stronger mechanical stimulus (Fig. 11D). These results support the idea that the medium pulse is a precursor of the large pulse. The medium pulse is assumed to be a subthreshold form of the large pulse.

The medium pulse was accompanied by a slow localized contraction of the cell body or by tilting of the cell body against the stalk. The tilting is caused by a localized contraction around the junction of the cell body and stalk. These localized contractions were easily overlooked, being too small for detection by the photoelectric contraction-detector employed.

On the basis on these results, we propose the hypothesis that the medium pulse is a mechanoreceptor potential activated by a contraction of the cell body in the same way as the large pulse. Since the contraction is small and graded, the resultant mechanoreceptor potential is small and graded.

When an inward current was injected into the cell body, the cell exhibited a depolarizing response superimposed on an exponential hyperpolarizing membrane potential shift (Fig. 16G). The depolarizing response resembled the medium pulse in its amplitude and time course and was accompanied by a localized contraction of the cell body. When the current intensity was increased, the depolarizing response was followed by a large pulse in association with an all-or-nothing cellular contraction. The large pulse was observed superimposed on the medium-pulse-like depolarizing response (Fig. 16I,J). These results imply that the inward-current-induced depolarizing response is a medium pulse evoked by a localized contraction of the cell body.

It should be noted that when a large pulse occurred during a medium pulse, summation of the two pulses was not observed (Figs 10B,C, 11C, 16I,J). In contrast, when a medium pulse occurred during a medium pulse, summation of the two pulses was observed (Fig. 1B). These results imply that an all-or-nothing cellular contraction is strong enough to activate almost all the mechanoreceptor channels on the cell body, so that a maximum, saturated mechanoreceptor current is evoked by the contraction. The peak of the large pulse corresponds to the membrane potential shifted by this saturated receptor current. Therefore, no change in the peak level of the large pulse is expected when a large pulse occurs during another change in the mechanoreceptor current-mediated membrane potential (the large or medium pulse). In contrast, a small localized contraction evokes an unsaturated mechanoreceptor current to produce a medium pulse, so that summation of two medium pulses is likely.

Involvement of Ca^{2+} in cellular contraction

We have confirmed the finding of previous investigators (Allen, 1973; Katoh and Naitoh, 1993) that *V. convallaria* contracted spontaneously or in response to mechanical stimulation in an EGTA-containing Ca^{2+}-deprived solution. It is well known that the contractile mechanism of vorticellid ciliates is activated by Ca^{2+} (Hoffmann-Berling, 1958; Townes and Brown, 1965; Amos, 1971, 1972; Routledge *et al.* 1975; Ochiai *et al.* 1979). It is therefore concluded that a mechanical stimulus applied to the cell body liberates Ca^{2+} from some intracellular Ca^{2+} storage sites. Recently Katoh and Kikuyama (1997) have demonstrated an all-or-nothing rise in the cytosolic Ca^{2+} concentration in response to mechanical stimulation of *Vorticella* sp. This all-or-nothing rise in Ca^{2+} concentration is assumed to be mediated by the Ca^{2+}-induced Ca^{2+} release mechanism (Endo *et al.* 1970; Katoh and Naitoh, 1993), which resides in intracellular Ca^{2+} storage sites, such as the endoplasmic reticulum and/or the membrane-bound microtubules (Amos, 1972; Allen, 1973; Routledge *et al.* 1975).

A small localized contraction is assumed to be caused by a slight localized increase in the cytosolic Ca^{2+} concentration following release of a small amount of Ca^{2+} from the storage site in response to a weak mechanical stimulus. The increased cytosolic Ca^{2+} concentration is insufficient to activate the Ca^{2+}-induced Ca^{2+} release mechanism. A stronger mechanical stimulus liberates larger amount of Ca^{2+} from the storage sites, causing an increase in the cytosolic Ca^{2+} concentration large enough to activate the Ca^{2+}-induced Ca^{2+} release mechanism. This regenerative, or all-or-nothing, increase in the Ca^{2+} concentration causes an all-or-nothing contraction.

We have demonstrated that an inward current injected into the cell body evoked a medium pulse and/or a large pulse, depending on its strength, in the presence of external Ca^{2+} (Fig. 16G–J). It is assumed that external Ca^{2+} is driven into the cell by the hyperpolarization. The entry of Ca^{2+} is small when the hyperpolarization is small, so that the resultant rise in the cytosolic Ca^{2+} concentration is insufficient to activate the Ca^{2+}-activated Ca^{2+} release mechanism, leading to only a small localized contraction. The Ca^{2+} entry is large when the hyperpolarization is large, so that the resultant rise in the cytosolic Ca^{2+} concentration is sufficient to activate the Ca^{2+}-induced Ca^{2+} release mechanism. An all-or-nothing release of Ca^{2+} occurs, and an all-or-nothing contraction results.

In this connection, it is interesting to note that *Didinium nasutum* discharges its extruding organelles in response to an injection of inward current in the presence of external Ca^{2+}. Ca^{2+} entering the cell activates the extrusion mechanism, and

this activates mechanoreceptor channels to produce a spike-like depolarizing response, which is seen superimposed on the exponential membrane hyperpolarization induced by the inward current (Hara *et al.* 1985).

Some other considerations

The peak level of the large pulse was observed to shift in the depolarizing direction with an increase in external Ca^{2+} concentration (Fig. 13B), while it did not shift with increases in external K^+ concentration (Fig. 13A). A change in the external Cl^- concentration did not affect the peak level. The reversal potential for the large pulse was approximately 33 mV (Figs 14, 15). These facts suggest the involvement of Ca^{2+} in the generation of the large pulse. However, a large pulse was observed even in an EGTA-containing solution with a very low Ca^{2+} concentration ($<10^{-8}$ mol l^{-1}), although its amplitude was small (data not shown). This suggests the involvement of cations other than Ca^{2+} in the generation of the large pulse, although the ion species remains unidentified.

Because of the low ionic strength of the external solution in which *V. convallaria* cell is bathed, surface charge neutralization is involved in the concentration effects of external cations on the membrane potential (Eckert and Brehm, 1979; Machemer, 1988). Since the surface charge properties of freshwater protozoa are unknown, we will not discuss further the effects of cations on the membrane potential of the cell in relation to its membrane ion permeability properties.

V. convallaria showed a depolarizing action potential when its membrane was depolarized to a level more positive than approximately 60 mV by an injection of outward current (Fig. 16C,D). Peristomal cilia showed ciliary reversal and/or ciliary stoppage in association with the action potential. It is therefore assumed that the action potential is caused by the activation of voltage-sensitive Ca^{2+} channels in the ciliary membrane in a similar manner to that in other ciliates. The rise in the Ca^{2+} concentration responsible for ciliary reversal caused by the Ca^{2+} action potential is confined to the cilia, so that it does not activate the contractile mechanism in the cell body (Naitoh, 1982; Machemer, 1988).

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References

ALLEN, R. D. (1973). Structures linking the myonemes, endoplasmic reticulum and surface membranes in the contractile ciliate *Vorticella*. *J. Cell Biol.* **56**, 559–579.

AMOS, W. B. (1971). Reversible mechanochemical cycle in the contraction of *Vorticella*. *Nature* **229**, 127–128.

AMOS, W. B. (1972). Structure and coiling of the stalk in the peritrich ciliates *Vorticella* and *Carchesium*. *J. Cell Sci.* **10**, 95–122.

ECKERT, R. AND BREHM, P. (1979). Ionic mechanism of excitation in *Paramecium*. *A. Rev. Biophys. Bioeng.* **8**, 353–383.

ENDO, M., TANAKA, M. AND OGAWA, Y. (1970). Calcium induced release of calcium from the sarcoplasmic reticulum of skinned skeletal muscle fibres. *Nature* **228**, 34–36.

HARA, R., ASAI, H. AND NAITOH, Y. (1985). Electrical responses of the carnivorous ciliate *Didinium nasutum* in relation to discharge of the extrusive organelles. *J. exp. Biol.* **119**, 211–224.

HODGKIN, A. L. AND HOROWICZ, P. (1957). Differential action of hypertonic solution on the twitch and action potential in single muscle fibre. *J. Physiol., Lond.* **136**, 17–18.

HOFFMANN-BERLING, H. (1958). Der Mechanismus eines neuen, von der Muskelkontraktion verschiedenen Kontraktionszyklus. *Biochim. biophys. Acta* **27**, 247–255.

JENNERICK, H. (1963). Phase plane trajectories of the muscle spike potential. *Biophys. J.* **3**, 363–377.

JONES, A. R., JAHN, T. L. AND FONSECA, J. R. (1970). Contraction of protoplasm. IV. Cinematographic analysis of the contraction of some peritriches. *J. cell. Physiol.* **75**, 9–20.

KATOH, K. AND KIKUYAMA, M. (1997). An all-or-nothing rise in cytosolic [Ca^{2+}] in *Vorticella* sp. *J. exp. Biol.* **200**, 35–40.

KATOH, K. AND NAITOH, Y. (1992). A mechanosensory mechanism for evoking a cellular contraction in *Vorticella*. *J. exp. Biol.* **168**, 253–267.

KATOH, K. AND NAITOH, Y. (1993). Control of cellular contraction by calcium in *Vorticella*. *J. exp. Biol.* **189**, 163–177.

MACHEMER, H. (1988). Electrophysiology. In Paramecium (ed. H.-D. Görtz), pp. 185–215. Berlin, Heidelberg, London, New York, Tokyo: Springer-Verlag.

MORETON, R. B. AND AMOS, W. B. (1979). Electrical recording from the contractile ciliate *Zoothamnium geniculatum* Ayrton. *J. exp. Biol.* **83**, 157–167.

NAITOH, Y. (1982). Protozoa. In *Electrical Conduction and Behaviour in 'Simple' Invertebrates* (ed. G. A. B. Shelton), pp. 1–48. Oxford: Clarendon Press.

NAITOH, Y. AND ECKERT, R. (1972). Electrophysiology of ciliate protozoa. In *Experiments in Physiology and Biochemistry* (ed. G. A. Kerkut), pp. 17–38. London, New York: Academic Press.

OCHIAI, T., ASAI, H. AND FUKUI, K. (1979). Hysteresis of contraction–extension cycle of glycerinated *Vorticella*. *J. Protozool.* **26**, 420–425.

ROUTLEDGE, L. M., AMOS, W. B., GUPTA, B. L., HALL, T. A. AND WEIS-FOGH, T. (1975). Microprobe measurements of calcium binding in the contractile spasmoneme of a vorticellid. *J. Cell Sci.* **19**, 195–201.

SHIONO, H., HARA, R. AND ASAI, H. (1980). Spontaneous membrane potential changes associated with the zooid and vacuolar contractions in *Vorticella convallaria*. *J. Protozool.* **27**, 83–87.

SHIONO, H. AND NAITOH, Y. (1977). Membrane potential change associated with a contraction of the cell body in *Vorticella convallaria*. *Zool. Mag. (Tokyo)* **86**, 408.

SHIONO, H. AND NAITOH, Y. (1978). Membrane potential change associated with a contraction of the cell body in *Vorticella convallaria*. II. The time relationship between contraction and membrane potential response. *Zool. Mag. (Tokyo)* **87**, 429.

SUGI, H. (1960). Propagation of contraction in the stalk muscle of *Carchesium*. *J. Fac. Sci. Univ. Tokyo Sect. IV* **8**, 603–615.

TOWNES, M. M. AND BROWN, D. E. S. (1965). The involvement of pH, adenosine triphosphate, calcium and magnesium in the contraction of glycerinated stalk of *Vorticella*. *J. cell. comp. Physiol.* **65**, 261–269.