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CONTROL OF CELLULAR CONTRACTION BY CALCIUM IN *VORTICELLA*

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Summary

1. *Vorticella* extracted with Triton X-100 contracted (i.e. the cell body shrank and the stalk coiled) when the external Ca^{2+} concentration was raised. The degree of contraction increased with increasing Ca^{2+} concentration.

2. The threshold Ca^{2+} concentration for shrinkage of the cell body was identical with that for coiling of the stalk in *Vorticella* extracted with Triton X-100.

3. Living *Vorticella* showed a graded shrinkage of the cell body when Ca^{2+} buffer was injected into the cell body, while the stalk showed coiling of an all-or-nothing type. The degree of shrinkage of the cell body increased with increasing free Ca^{2+} concentration of the buffer.

4. Living *Vorticella* showed a sustained contraction in response to external application or intracellular injection of caffeine. The effect of caffeine was inhibited by intracellular injection of procaine or Ruthenium Red.

5. *Vorticella* injected with Ruthenium Red showed graded shrinkage of the cell body as well as graded coiling of the stalk when Ca^{2+} buffer was injected into the cell body.

6. Caffeine, procaine and Ruthenium Red had no measurable effect on Ca^{2+}-activated contraction in *Vorticella* extracted with Triton X-100.

7. It is assumed that regenerative liberation of Ca^{2+} from the endoplasmic reticulum and/or membranous tubules in the contractile system (Ca^{2+}-induced Ca^{2+} release) is responsible for evoking contraction of an all-or-nothing type following stimulation in living *Vorticella*.

Introduction

The sessile peritrich ciliate *Vorticella* exhibits contraction of an all-or-nothing type involving shrinkage of the cell body and coiling of the stalk in response to mechanical stimulation. As early as 1958, Hoffmann-Berling found that the stalk of a glycerol-extracted *Vorticella* coiled when Ca^{2+} was added to the external solution and that it uncoiled when Ca^{2+} was removed (see also Amos *et al.* 1976). Ochiai *et al.* (1983) applied Ca^{2+} electrophoretically to a localized portion of the stalk of a glycerol-extracted *Vorticella* and found that only the portion subjected to Ca^{2+} showed coiling. These results suggest the presence of a mechanism for evoking a regenerative increase in intracellular

Key words: *Vorticella*, cellular contraction, Ca^{2+}-induced Ca^{2+} release.

Ca^{2+} concentration which, in turn, is responsible for an all-or-nothing cellular contraction in living *Vorticella*.

Allen (1973) reported that external Ca^{2+} was not required for cellular contraction in living specimens of *Vorticella*. Katoh and Naitoh (1991, 1992*a*) found that the mechanical threshold for evoking a contraction in *Vorticella* was not affected by removal of Ca^{2+} from the external solution. These results imply that the Ca^{2+} required for the contraction comes from intracellular Ca^{2+} storage sites and not from the external solution.

In their electron microscopical examinations of vorticellid ciliates, several authors have reported that membranous tubules (tubular endoplasmic reticulum) are present in the spasmoneme, which is responsible for the coiling of the stalk, and in the myoneme, which is responsible for the shrinkage of the cell body, and that endoplasmic reticulum is present around the myoneme (Carasso and Favard, 1966; Allen, 1973; Amos, 1972; Amos *et al.* 1976). Moreover, Carasso and Favard (1966) demonstrated the presence of calcium in the tubules and the endoplasmic reticulum by cytochemical methods. These membranous systems are, therefore, strong candidates for the Ca^{2+} storage sites.

We recently suggested the possibility that the Ca^{2+}-induced Ca^{2+} release mechanism, which was first reported by Endo *et al.* (1970) (see also Ford and Podolsky, 1970) in the sarcoplasmic reticulum of skeletal muscle fibres, might cause a regenerative increase in the intracellular Ca^{2+} concentration responsible for the all-or-nothing type of contraction in *Vorticella* (Katoh and Naitoh, 1992*a*). In the research described in the present paper, we accordingly determined the effects of Ca^{2+} and of drugs that affect the liberation of Ca^{2+} from the sarcoplasmic reticulum of the skeletal muscle fibre (Endo, 1977; Lai *et al.* 1988) on the contractile activity of both living and Triton-extracted specimens of *Vorticella*. Some of these results have been presented verbally elsewhere (Katoh and Naitoh, 1991, 1992*b*).

Materials and methods

Specimens of *Vorticella* sp. were grown at 20 °C on a glass slide in a bacterized saline solution (0.1 mmol l^{-1} KCl, 0.09 mmol l^{-1} $CaCl_2$ and 0.1 mmol l^{-1} $MgSO_4$ final concentration) containing 0.1 % (w/w) dehydrated cereal leaves (Sigma). Specimens were washed with a standard saline solution, which contained 1 mmol l^{-1} KCl, 1 mmol l^{-1} $CaCl_2$ and 10 mmol l^{-1} Tris–maleate buffer (pH 7.0), and then immersed in the same solution for approximately 10 min prior to experimentation.

Detergent-extraction of the specimens of *Vorticella* was performed according to Naitoh and Kaneko (1972). Washed specimens attached to a glass slide were kept immersed for approximately 1 min in the extraction medium, which consisted of 0.1 % (v/v) octylphenoxypolyethoxyethanol (Triton X-100, Wako Pure Chemicals Co., Tokyo), 20 mmol l^{-1} KCl, 10 mmol l^{-1} EDTA and 10 mmol l^{-1} Tris–maleate buffer (pH 7.0). The extracted specimens were then washed gently three times with a washing medium, which consisted of 50 mmol l^{-1} KCl, 2 mmol l^{-1} EGTA and 10 mmol l^{-1} Tris–maleate buffer (pH 7.0), and kept in this medium for 15 min to remove Triton X-100. Washed extracted specimens were immersed in reactivation media with differing Ca^{2+} concentrations. The

reactivation medium consisted of 50 mmol l^{-1} KCl, 10 mmol l^{-1} Tris–maleate buffer (pH 7.0) and calcium buffer with 2 mmol l^{-1} EGTA.

In order to change cytoplasmic Ca^{2+} concentration of a living specimen, Ca^{2+} buffer solution was injected into the cell body according to the method of Hiramoto (1974). A specimen was first detached from its substratum (a glass slide) and its cell body was fixed at the tip of a suction pipette (about 5 μm in inner diameter) by lowering the hydrostatic pressure inside the pipette. The tip of a micropipette (1–2 μm in inner diameter) for injection was then inserted into the cell body. Ca^{2+} buffer solutions with different Ca^{2+} concentrations were prepared by mixing 100 mmol l^{-1} EGTA and 100 mmol l^{-1} calcium-bound EGTA in various ratios. The pH of the Ca^{2+} buffers was adjusted to 7.0 with 100 mmol l^{-1} Pipes–KOH buffer. The volume of Ca^{2+} buffer injected into the cell body was approximately 3.5 pl, which corresponded to about 10 % of the volume of the cell body and was large enough to keep the cytoplasmic free Ca^{2+} concentration at a value identical with that for the Ca^{2+} buffer. The external solution for the injection experiments was a mixture of 1 mmol l^{-1} KCl, 1 mmol l^{-1} EGTA and 10 mmol l^{-1} Tris–maleate buffer (pH 7.0).

Caffeine, ryanodine, procaine, Ruthenium Red (all from Wako Pure Chemicals Co., Tokyo) and Ins(1,4,5)P_3 (D-myo-inositol 1,4,5-trisphosphate; Boehringer GmbH, Mannheim) were dissolved in solutions containing 100 mmol l^{-1} KCl, 0.02 mmol l^{-1} EGTA and 5 mmol l^{-1} Pipes–KOH buffer (pH 7.0), which were used for intracellular injection. The amount of solution injected into the cell body was approximately 3.5 pl. These drugs were dissolved in a solution containing 1 mmol l^{-1} KCl, 1 mmol l^{-1} EGTA and 10 mmol l^{-1} Tris–maleate buffer (pH 7.0) for external application.

A mechanical stimulus was applied to the cell body with a microneedle driven by a piezoelectric transducer according to Katoh and Naitoh (1992*a*) (see also Naitoh and Eckert, 1969). Magnified images (approximately ×200) of the specimens were recorded on a video tape, and the specimens' contractile responses were examined on the displayed pictures. All the experiments were performed at room temperature (20–23 ˚C).

Results

Effect of Ca^{2+} concentration on Triton-extracted specimens

Triton-extracted specimens of *Vorticella* exhibited a contraction (shrinkage of the cell body and coiling of the stalk) when the free Ca^{2+} concentration in the reactivation medium was raised above a threshold value of approximately 8.9×10^{-8} mol l^{-1}. Fig. 1 shows a representative series of photographs of a single extracted specimen in five different reactivation media with different free Ca^{2+} concentrations ranging from 8.9×10^{-8} to 1.9×10^{-6} mol l^{-1}.

The relationship between the degree of contraction and the free Ca^{2+} concentration of the reactivation medium is shown in Fig. 2. The degree of shrinkage of the cell body is expressed as the decrease in the length of the longitudinal (oralo-aboral) axis of the cell body relative to the maximum decrease in the length at a Ca^{2+} concentration higher than 1.9×10^{-6} mol l^{-1}($\Delta Lc/\Delta Lc_{max}$; see the inset of Fig. 2). Similarly, the degree of coiling of the stalk is expressed as the decrease in the distance between the ends of the stalk relative

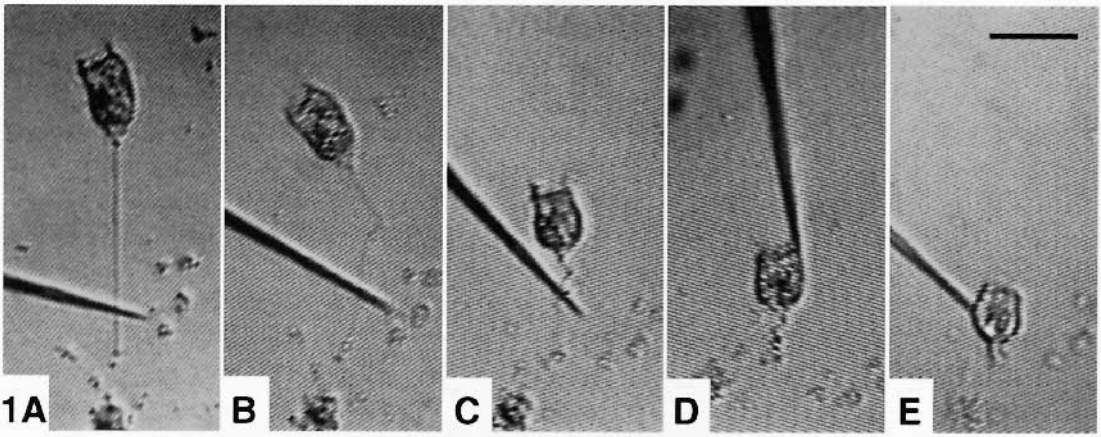


Fig. 1. A representative series of photographs of a single specimen of *Vorticella* sp. extracted with Triton X-100 in five different reactivation media with different free Ca^{2+} concentrations. Free Ca^{2+} concentrations were: A, 8.9×10^{-8} mol l^{-1}; B, 1.4×10^{-7} mol l^{-1}; C, 2.1×10^{-7} mol l^{-1}; D, 3.1×10^{-7} mol l^{-1}; E, 1.9×10^{-6} mol l^{-1}. A microneedle, which is seen as a tapered black bar in each photograph, was used to keep the specimen within the focal plane of the microscope objective (×20). The specimen was videorecorded and displayed on the monitor screen for photography. Scale bar, 50 μm.

to its maximum decrease at a Ca^{2+} concentration higher than 1.9×10^{-6} mol l^{-1} ($\Delta Ls/\Delta Ls_{max}$; see the inset of Fig. 2). Both values increased sigmoidally with a logarithmic increase in the Ca^{2+} concentration, and reached 100 % at approximately 1.9×10^{-6} mol l^{-1} Ca^{2+}. The Ca^{2+} concentration corresponding to 50 % contraction was approximately 1.9×10^{-7} mol l^{-1}.

Injection of Ca^{2+} buffer into the cell body of living Vorticella

We examined the contractile responses of living specimens of *Vorticella* to injection of Ca^{2+} buffer solution into the cell body. Fig. 3 shows three representative pairs of photographs of three different specimens into which were injected Ca^{2+} buffer solutions with different free Ca^{2+} concentrations.

The relationships between the degree of contraction and free Ca^{2+} concentration of injected buffer solution are shown in Fig. 4. Neither coiling of the stalk nor shrinkage of the cell body was evoked when the free Ca^{2+} concentration was lower than 5.1×10^{-8} mol l^{-1} (corresponding to Fig. 3Aii). When the free Ca^{2+} concentration was slightly higher than 5.1×10^{-8} mol l^{-1} the stalk showed more than 79 % of the maximum coiling, whereas the cell body remained relaxed (corresponding to Fig. 3Bii). When the free Ca^{2+} concentration was further increased the cell body started to show shrinkage. The degree of shrinkage increased with increasing Ca^{2+} concentration, reaching its maximum value (100 %) at 2.0×10^{-7} mol l^{-1}. The degree of coiling of the stalk was also 100 % at this Ca^{2+} concentration (corresponding to Fig. 3Cii). The Ca^{2+} concentrations that corresponded to 50 % contraction were approximately 5.1×10^{-8} mol l^{-1} for coiling of the stalk and 1.4×10^{-7} mol l^{-1} for shrinkage of the cell body.

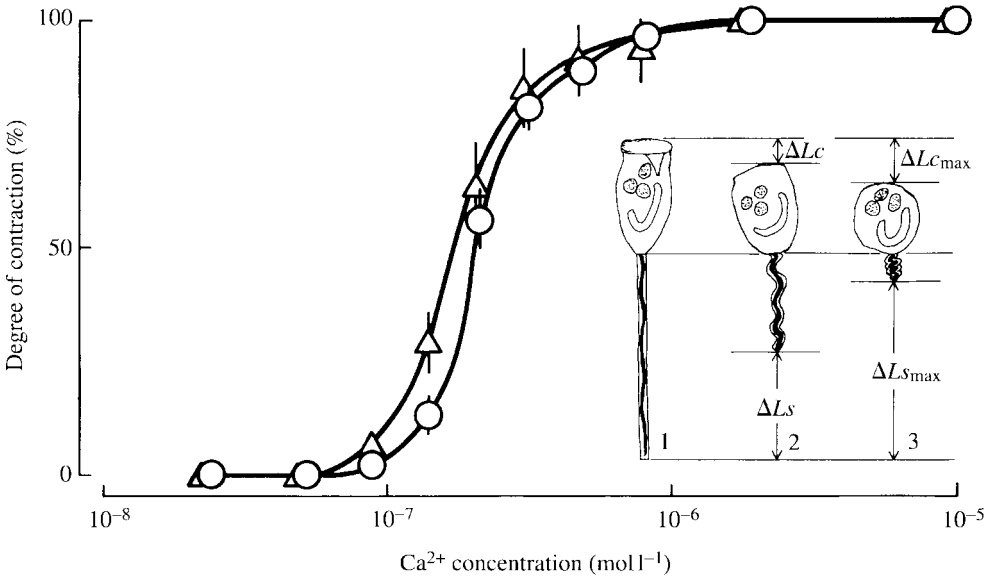


Fig. 2. The relationship between the degree of contraction (shrinkage of the cell body, triangles; coiling of the stalk, circles) and free Ca^{2+} concentration in the reactivation medium for specimens of *Vorticella* sp. extracted in Triton X-100. Each symbol is the mean (±S.E.M.) of five measurements using different specimens. Error bars are omitted when they are smaller than the symbol. Note that some symbols have been offset slightly for clarity. The lines of best fit were drawn by eye. Inset, a diagram showing *Vorticella* in a state of full extension (1), in a state of half contraction (2) and in a state of full contraction (3). ΔLc, the change in the length of the longitudinal axis of the cell body; ΔLc_{max}, the maximum change in the length of the longitudinal axis of the cell body; ΔLs, the change in the distance between the ends of the stalk; ΔLs_{max}, the maximum change in the distance between the ends of the stalk. The degree of shrinkage of the cell body and that of coiling of the stalk are defined as $\Delta Lc/\Delta Lc_{max}$ and $\Delta Ls/\Delta Ls_{max}$, respectively.

Effects of drugs on the degree of contraction

In this series of experiments, we examined the effects of drugs that affect Ca^{2+} release from the sarcoplasmic reticulum in skeletal muscle fibres on the contractile activities of both living and Triton-extracted specimens of *Vorticella*.

Caffeine

Living specimens of *Vorticella* exhibited spontaneous contractions at very low frequency ($0.1\pm0.09\,min^{-1}$) in the standard saline solution (the reference solution). The frequency of contraction, which was determined for successive 1 min periods, increased suddenly when caffeine was introduced into the standard saline solution at a final concentration of $25\,mmol\,l^{-1}$ (Fig. 5). The frequency decreased gradually with time, reaching a steady value that was significantly ($P<0.05$) higher than that before application of caffeine in about 7 min. In contrast, the duration of contraction, which was defined as the period from the beginning of contraction to the time when the stalk had uncoiled by

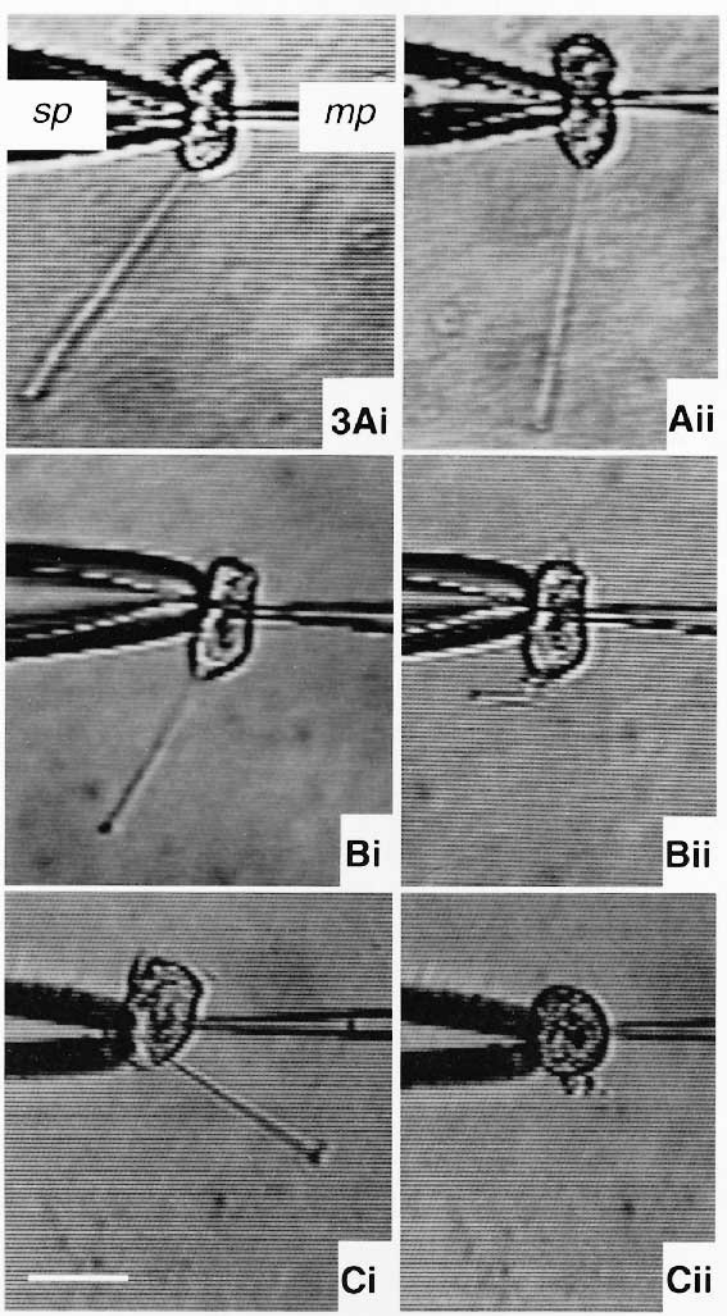


Fig. 3. Representative pairs of photographs of three living specimens of *Vorticella* sp. into which were injected Ca^{2+} buffer solutions containing different Ca^{2+} concentrations. The specimens were videorecorded and displayed on the monitor screen for photography. *sp*, a suction pipette to fix the specimen in the visual field of the microscope; *mp*, a micropipette for injection of Ca^{2+} buffer solution into the cell body; (Ai, Bi, Ci) pictures taken before injection of Ca^{2+} buffer; (Aii, Bii, Cii) pictures taken after injection of Ca^{2+} buffer. The free Ca^{2+} concentrations in the injected buffer were: A, 2.3×10^{-8} mol l^{-1}; B, 8.9×10^{-8} mol l^{-1}; C, 1.9×10^{-6} mol l^{-1}.

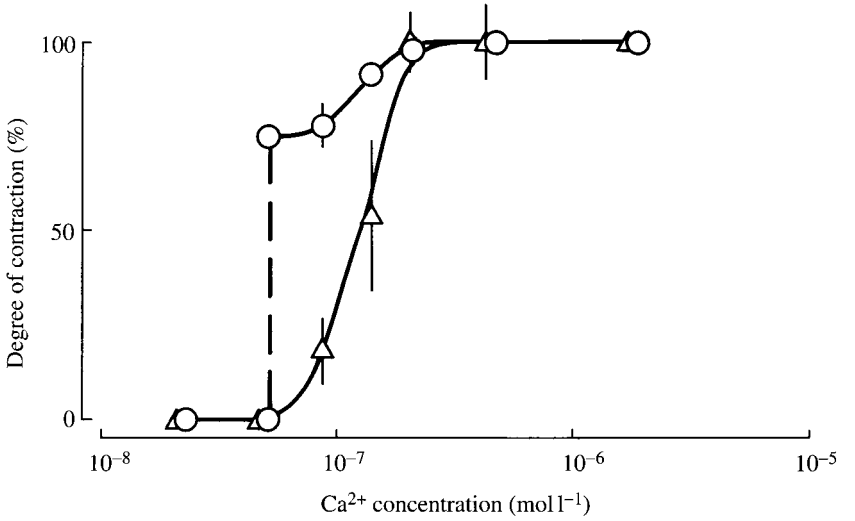


Fig. 4. The relationship between the degree of contraction and the free Ca^{2+} concentration in Ca^{2+} buffer solution injected into the cell body of living specimens of *Vorticella* sp. See the legend of Fig. 2 for calculation of the degree of contraction. Triangles, the degree of shrinkage of the cell body; circles, the degree of coiling of the stalk. Each symbol is the mean (±S.E.M.) of five measurement using different specimens. Error bars are omitted when they are smaller than the symbol. Note that some symbols have been offset slightly for clarity. The lines of best fit were drawn by eye.

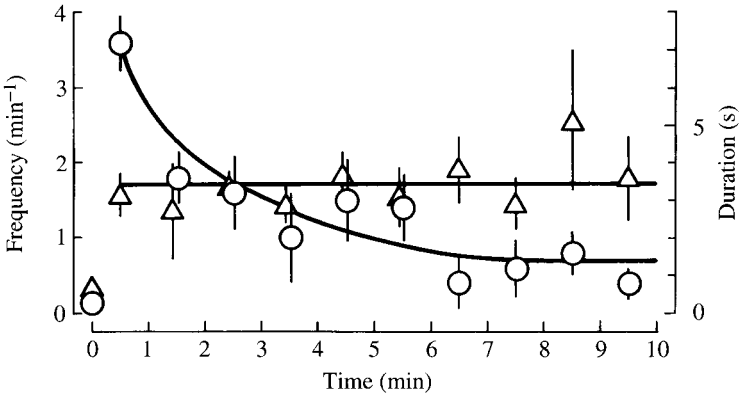


Fig. 5. Time courses of change in the frequency (circles) and in the duration (triangles) of spontaneous contractions in living specimens of *Vorticella* sp. after their immersion into a solution containing 25 mmol l^{-1} caffeine. The frequency is presented as the number of contractions in successive 1 min periods. The duration was defined as the period from the beginning of contraction to the time when the stalk had uncoiled by 80 % of the maximum degree of coiling. Symbols at time zero correspond to the values obtained in a solution without caffeine. Each symbol is the mean (±S.E.M.) of nine measurements using different specimens. Error bars are omitted when they are smaller than the symbol. Note that some symbols have been offset slightly for clarity. The lines of best fit were drawn by eye.

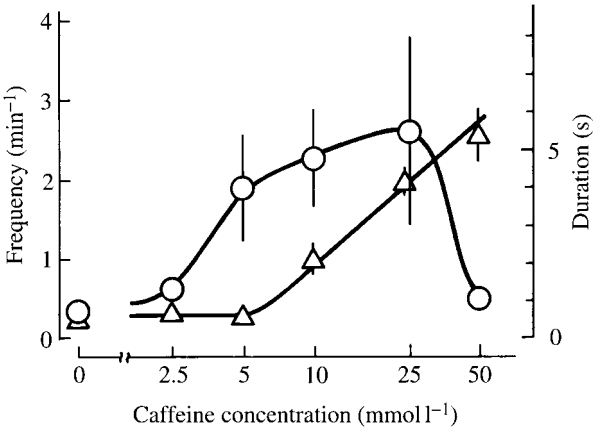


Fig. 6. Effects of the concentration of caffeine in the external solution on the frequency (circles) and the duration (triangles) of spontaneous contractions in living specimens of *Vorticella* sp. The frequency was obtained from the number of contractions exhibited by the specimens in the 3 min after their immersion into each caffeine-containing solution. See the legend of Fig. 5 for the definition of the duration of contraction. Each symbol is the mean (±S.E.M.) of six measurements using different specimens. Error bars are omitted when they are smaller than the symbol. Note that some symbols have been offset slightly for clarity. The lines of best fit were drawn by eye.

80 % of the maximum degree of coiling, increased immediately after application of caffeine and remained unchanged for more than 10 min. The increase in the duration was mostly due to prolongation of the relaxation phase of the contraction.

To examine the effects of caffeine concentration more precisely, both the frequency and the duration of contraction were determined by averaging the values for 3 min after administration of caffeine at different concentrations ranging from 2.5 to 50 mmol l^{-1}. As shown in Fig. 6, both values increased with increasing caffeine concentration above respective threshold values (approximately 2.5 mmol l^{-1} for frequency and 5 mmol l^{-1} for duration). The frequency decreased when caffeine concentration was more than 50 mmol l^{-1}.

An injection of a saline solution containing 50 mmol l^{-1} caffeine into the cell body of a living specimen caused a sudden increase in the frequency of contraction to 3.0±0.9 min^{-1} (N=7); a value significantly (P<0.025) higher than that measured immediately after an injection of the caffeine-free reference solution (0.57±0.18 min^{-1}, N=7). In contrast, the duration of contraction was relatively unaffected by the injection of caffeine (control, 0.85±0.15 s, N=4; caffeine-injected, 0.90±0.12 s, N=5). In contrast to living specimens, administration of caffeine into reactivation medium did not produce contraction in specimens extracted in Triton X-100.

Ryanodine

Ryanodine did not affect the contraction of living specimens of *Vorticella* when it was applied externally (1–10 μmol l^{-1}), but its injection into the cell body (2 μmol l^{-1})

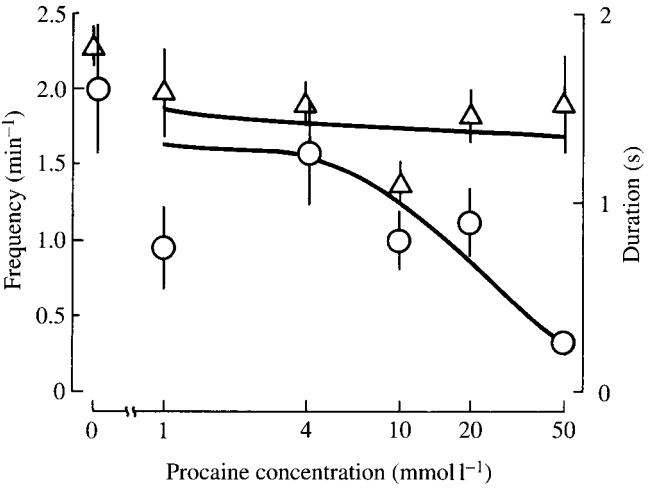


Fig. 7. Effects of the concentration of procaine in the external solution on the frequency (circles) and the duration (triangles) of contraction in living specimens of *Vorticella* sp. bathed in a solution containing 10 mmol l^{-1} caffeine. See the legend of Fig. 5 for the definition of the duration of contraction and that of Fig. 6 for the definition of the frequency of contraction. Each symbol is the mean (±S.E.M.) of seven measurements using different specimens. Error bars are omitted when they are smaller than the symbol. Note that symbols have been offset slightly for clarity. The lines of best fit were drawn by eye.

caused a sudden increase in the frequency of contraction to 0.56±0.06 min^{-1} (N=8), a value significantly (P<0.001) greater than that obtained immediately after an injection of the ryanodine-free reference solution (0.19±0.06 min^{-1}, N=7). In contrast, administration of ryanodine into the reactivation medium (1–10 μmol l^{-1}) did not produce contraction in Triton-extracted specimens.

Inositol 1,4,5-trisphosphate

Neither external application (100 μmol l^{-1}) nor injection (100 μmol l^{-1}) of InsP_3 affected the frequency or duration of contraction. InsP_3 (2–100 μmol l^{-1}) did not affect the Ca^{2+}-induced contraction of Triton-extracted specimens.

Procaine

We examined the effects of procaine on the caffeine-induced contraction. As shown in Fig. 7, external application of procaine (1–50 mmol l^{-1}) suppressed the increase in the frequency of contraction expected to be evoked by 10 mmol l^{-1} caffeine in living specimens of *Vorticella*. Procaine slightly affected the duration of contraction.

A mechanical stimulus given to the cell body of living specimens failed to evoke a cellular contraction in the presence of 50 mmol l^{-1} procaine. The inhibitory effect of procaine disappeared 10–20 min after removal of the drug from the external solution.

Injection of a solution containing 5 mmol l^{-1} procaine into the cell body of living specimens of *Vorticella* suppressed an increase in the frequency of contraction evoked by

Fig. 8. Representative pairs of photographs of three living specimens of *Vorticella* sp. preinjected with Ruthenium Red into which Ca^{2+} buffer solutions with different free Ca^{2+} concentrations were injected. The specimens were videorecorded and displayed on the monitor screen for photography. *sp*, a suction pipette to fix the specimen in the visual field on the microscope; *mp*, a micropipette for injecting both Ruthenium-Red-containing solution and Ca^{2+} buffer solution into the cell body of the specimen; (Ai, Bi, Ci) photographs taken after injection of the solution containing Ruthenium Red, before Ca^{2+} buffer was injected; (Aii, Bii, Cii) photographs taken after injection of Ca^{2+} buffer. The free Ca^{2+} concentrations in the injected buffer were: A, 1.4×10^{-7} mol l^{-1}; B, 2.1×10^{-7} mol l^{-1} and C, 4.8×10^{-7} mol l^{-1}. *o*, a drop of injected oil, which was used to separate Ca^{2+} buffer solution from Ruthenium Red solution in the pipette. Scale bar, 50 μm.

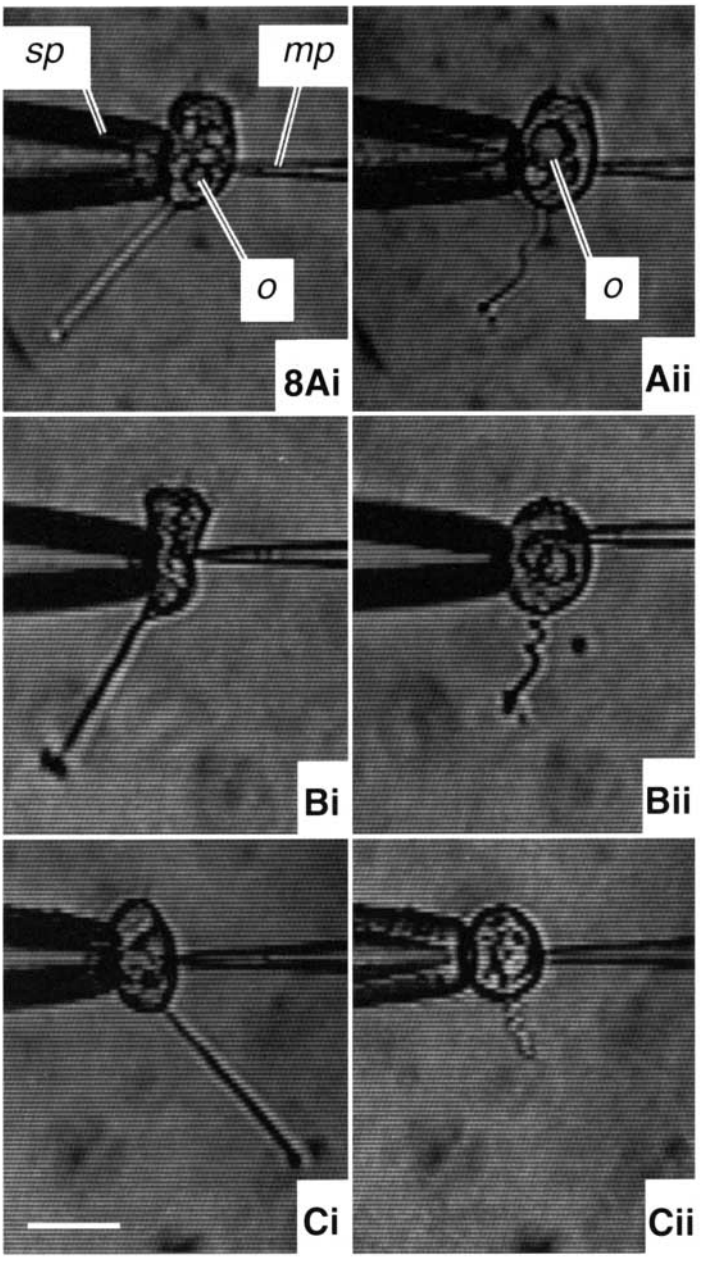
external application of 10 mmol l^{-1} caffeine. Specimens injected with procaine did not contract in response to mechanical stimulation. In contrast to living specimens, procaine had no effects on Ca^{2+}-evoked contraction in Triton-extracted specimens.

Ruthenium Red

External application of Ruthenium Red (1–500 μmol l^{-1}) did not affect the caffeine-evoked increase in contraction frequency in living specimens. Living specimens contracted in response to a mechanical stimulus applied to their cell body in the presence of Ruthenium Red in the external solution (1–500 μmol l^{-1}). In contrast, intracellular injection of a solution containing 1 μmol l^{-1} Ruthenium Red suppressed the caffeine-evoked increase in the frequency of contraction in living specimens. Specimens injected with Ruthenium Red failed to show a contraction in response to mechanical stimulation.

In the next series of experiments, a solution containing 1 μmol l^{-1} Ruthenium Red was first injected, and a Ca^{2+} buffer solution was subsequently injected, into the cell body of a living specimen of *Vorticella*. When a Ca^{2+} buffer solution with a free Ca^{2+} concentration of 8.9×10^{-8} mol l^{-1} was injected within 3 min of Ruthenium Red injection, the stalk showed maximum coiling (98.3±3.8 %, N=3), while the cell body shrank by only 13.8±6.3 % (N=3). When the same buffer solution was injected more than 5 min after Ruthenium Red injection, the degree of coiling of the stalk was as low as that of the cell body (14.5±5.1 %, N=6, for the stalk; 20.9±3.9 %, N=6, for the cell body). Fig. 8 shows three representative pairs of photographs of three different specimens of *Vorticella* into which Ca^{2+} buffer solutions with different free Ca^{2+} concentrations (A, 1.4×10^{-7} mol l^{-1}; B, 2.1×10^{-7} mol l^{-1}; C, 4.8×10^{-7} mol l^{-1}) had been injected 5 min after preinjection of Ruthenium Red.

The relationship between the degree of contraction and free Ca^{2+} concentration of the injected Ca^{2+} buffer solution is shown in Fig. 9. The degree of shrinkage of the cell body and of coiling of the stalk increased sigmoidally with logarithmic increases in free Ca^{2+} concentration. The Ca^{2+} concentration that corresponded to 50 % contraction was approximately 1.4×10^{-7} mol l^{-1}. In contrast to living specimens, administration of 1–10 μmol l^{-1} Ruthenium Red into the reactivation medium had no effect on Ca^{2+}-evoked contraction in Triton-extracted specimens. Similar results were obtained when Ca^{2+} buffer solutions were injected into procaine-injected specimens (data not shown).



Discussion

Triton-extracted specimens of *Vorticella* exhibited shrinkage of the cell body and coiling of the stalk when the free Ca^{2+} concentration of the reactivation medium was raised above approximately 8.9×10^{-8} mol l^{-1}. Shrinkage of the cell body is caused by Ca^{2+}-mediated contraction of the myoneme and coiling of the stalk is caused by Ca^{2+}-mediated contraction of the spasmoneme (Allen, 1973; Amos *et al.* 1976). The degree of

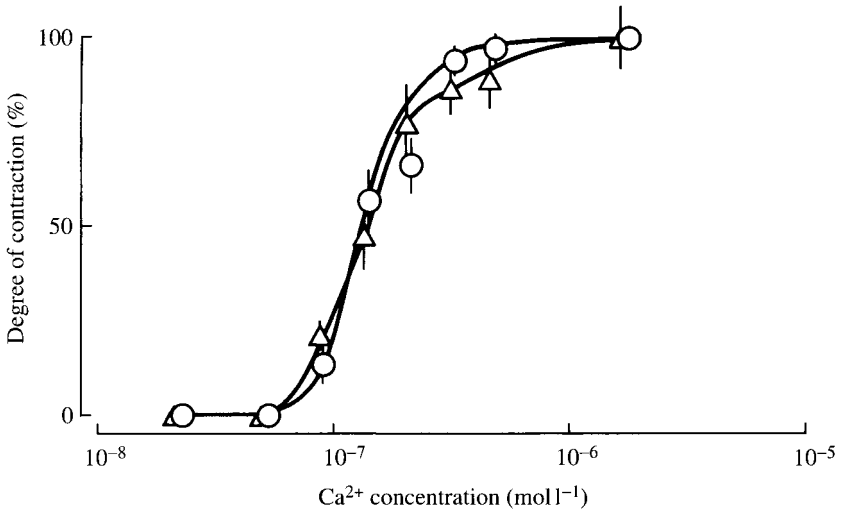


Fig. 9. The relationship between the degree of contraction and free Ca^{2+} concentration in the Ca^{2+} buffer solution injected into the cell body of Ruthenium-Red-preinjected living specimens of *Vorticella* sp. See the legend of Fig. 2 for calculation of the degree of contraction. Triangles, the degree of shrinkage of the cell body; circles, the degree of coiling of the stalk. Each symbol is the mean (±S.E.M.) of six measurements using different specimens. Error bars are omitted when they are smaller than the symbol. Note that some symbols have been offset slightly for clarity. The lines of best fit were drawn by eye.

contraction increased with increasing free Ca^{2+} concentration (Figs 1, 2). The relationship between free Ca^{2+} concentration and the degree of shrinkage of the cell body was essentially identical to that between free Ca^{2+} concentration and the degree of coiling of the stalk (Fig. 2). This indicates that the Ca^{2+} sensitivity of the myoneme is almost identical with that of the spasmoneme.

In contrast to Triton-extracted *Vorticella*, living *Vorticella* showed spontaneous or mechanically evoked all-or-nothing contractions. We therefore assumed that a regenerative increase in the intracellular Ca^{2+} concentration is involved in the contraction of living *Vorticella*. Moreton and Amos (1979) proposed that an influx of Ca^{2+} into the cell from the external solution, accompanied by a regenerative Ca^{2+} action potential, was responsible for the regenerative increase in the intracellular Ca^{2+} concentration in *Zoothamnium geniculatum.* This hypothesis is, however, not applicable to *Vorticella*, for contraction of *Vorticella* occurs in the absence of external Ca^{2+} (Allen, 1973; Katoh and Naitoh, 1992*a*). Katoh and Naitoh (1992*a*) therefore suggested that a Ca^{2+}-induced Ca^{2+} release mechanism, first observed in the sarcoplasmic reticulum of skeletal muscle fibres by Endo *et al.* (1970) (see also Ford and Podolsky, 1970) was involved in the regenerative increase in the intracellular Ca^{2+} concentration in *Vorticella*.

As shown in Figs 3 and 4, injection of Ca^{2+} buffer solution with a free Ca^{2+} concentration of approximately 5.1×10^{-8} mol l^{-1} [a value lower than the threshold for evoking contraction in Triton-extracted specimens (approximately 8.3×10^{-8} mol l^{-1}, see

Fig. 2)] into the cell body of a living *Vorticella* produced nearly maximal coiling of the stalk, but it did not produce shrinkage of the cell body. It is therefore assumed that a mechanism that causes a regenerative increase in the free Ca^{2+} concentration in the stalk is activated by injection of Ca^{2+} buffer into the cell body. It is interesting to note that, when the cell body was stimulated in a living *Carchecium polypinum*, a relative of *Vorticella*, coiling of the stalk started from the junction between the cell body and the stalk and propagated down the stalk (Sugi, 1960). The Ca^{2+} required for coiling of the stalk must be supplied from some Ca^{2+} storage sites in the stalk, because the external solution for the injection experiments contains $1\,mmol\,l^{-1}$ EGTA, so its free Ca^{2+} concentration is less than $10^{-9}\,mol\,l^{-1}$.

A similar mechanism that causes a regenerative rise in the Ca^{2+} concentration in the cell body is assumed to be activated by injection of the Ca^{2+} buffer. However, the buffer action is strong enough to keep the Ca^{2+} concentration in the cell body below the threshold for shrinkage. Ca^{2+} buffer injected into the cell body is assumed to diffuse only slightly into the stalk, because the stalk is thin (approximately $3.4\,\mu m$ in inner diameter) and long (more than $100\,\mu m$). Therefore, a regenerative increase in Ca^{2+} concentration can take place in the stalk. The degree of coiling evoked by injection of Ca^{2+} buffer into the cell body was always slightly smaller than its maximum value (Fig. 4). This must be attributable to a slight diffusion of Ca^{2+} buffer into the stalk from the cell body.

Caffeine can produce a contracture of skeletal muscle fibres. The contracture is attributable to a sustained increase in the sarcoplasmic Ca^{2+} concentration, caused by caffeine-induced Ca^{2+} release from the sarcoplasmic reticulum (Endo *et al.* 1970). External application of caffeine caused an increase in the frequency and the duration of contraction in living *Vorticella* (Fig. 6). When the final concentration was more than $50\,mmol\,l^{-1}$, it even caused a sustained contraction, which corresponds to the contracture of skeletal muscle fibres. An intracellular injection of caffeine also produced an increase in the frequency of contraction. All the caffeine experiments were performed in Ca^{2+}-free solution containing EGTA. The Ca^{2+} required to evoke contraction is, therefore, presumed to be supplied from some intracellular Ca^{2+} storage sites. In contrast to living *Vorticella*, Triton-extracted *Vorticella* did not show a contraction following administration of caffeine into their reactivation medium.

Procaine and Ruthenium Red are known to inhibit the Ca^{2+}-induced Ca^{2+} release from sarcoplasmic reticulum (Endo, 1977; Volpe *et al.* 1986). Living *Vorticella* immersed or injected with a solution containing procaine showed neither an increase in the frequency of contraction in response to caffeine nor a contraction in response to mechanical stimulation.

Living *Vorticella* injected with Ruthenium Red showed neither an increase in the frequency of contraction in response to caffeine nor a contraction in response to mechanical stimulation. However, living *Vorticella* injected with Ruthenium Red showed contraction upon injection of a Ca^{2+} buffer solution with a free Ca^{2+} concentration higher than the threshold for evoking contraction in Triton-extracted *Vorticella*. The contraction was not of an all-or-nothing type, but graded. The degree of contraction was dependent on the Ca^{2+} concentration of the injected buffer. The relationship between the free Ca^{2+}

concentration and the degree of contraction was identical with that for Triton-extracted specimens (compare Fig. 9 with Fig. 2). This indicates that the contractile activities of the spasmoneme and the myoneme are not affected by Ruthenium Red. This was also shown by the observation that the presence of Ruthenium Red in the reactivation medium did not affect the Ca^{2+}-activated contraction in the Triton-extracted specimens.

The present findings strongly support the idea that the regenerative increase in the intracellular free Ca^{2+} concentration responsible for the all-or-nothing contractions of living *Vorticella* is mediated by Ca^{2+}-induced Ca^{2+} release from some membrane-bound system that is similar in its characteristics to the Ca^{2+}-induced Ca^{2+} release reported in the sarcoplasmic reticulum of skeletal and cardiac muscle (Endo, 1977) and the endoplasmic reticulum of nerve cells (Neering and McBurney, 1984). Recently, InsP_3-mediated Ca^{2+}-induced Ca^{2+} release mechanisms have been reported in sarcoplasmic reticulum of smooth muscle fibres, in the endoplasmic reticulum of some egg cells and in endoplasmic reticulum vesicles from cerebellum incorporated into planar bilayers (Iino and Endo, 1992; Miyazaki *et al.* 1992; Bezprozvanny *et al.* 1991). *Vorticella* did not show contraction in response to an injection of InsP_3.

Several authors have reported that membranous tubules (tubular endoplasmic reticulum) are present in the spasmoneme and the myoneme of *Vorticella* and that endoplasmic reticulum is present around the myoneme (Carasso and Favard, 1966; Allen, 1973). The Ca^{2+}-induced Ca^{2+} release mechanism probably resides in these reticular systems in living *Vorticella*.

We found that a protozoan, *Vorticella*, utilizes its endoplasmic reticulum for the control of its Ca^{2+}-dependent contractile activity in a way similar to the skeletal muscle fibre, although its contractile mechanism is very different from that of the skeletal muscle fibre. This finding is important for understanding the evolution and differentiation of mechanisms of cellular contractile activity in the animal kingdom.

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