J. exp. Biol. 168, 253–267 (1992)
Printed in Great Britain © The Company of Biologists Limited 1992

A MECHANOSENSORY MECHANISM FOR EVOKING CELLULAR CONTRACTION IN *VORTICELLA*

BY KAORU KATOH AND YUTAKA NAITOH*

Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305, Japan

Accepted 11 March 1992

Summary

1. *Vorticella* contracted (i.e. shrinkage of the cell body and coiling of the stalk) in response to being touched with a microneedle.

2. The threshold excursion of the microneedle required to evoke a contraction was smallest on the cell body. On the stalk, it was larger in regions farther from the cell body.

3. Hitting the stalk did not evoke a contraction if the stalk was mechanically clamped in a region between the site of the hit and the cell body.

4. Rapidly drawing a small portion of the cell body into a micropipette by suction evoked a contraction, whereas a similar stimulus applied to the stalk did not.

5. The threshold depression of the surface membrane of the cell body required to evoke a contraction was inversely proportional to the rate of depression.

6. Tilting the stalk of a specimen detached from its substratum evoked a contraction. The threshold degree of tilting was inversely proportional to the angular velocity of tilting.

7. Tilting the stalk is assumed to cause a localized depression of the surface membrane of the cell body around the stalk.

8. We concluded (1) that the cell body is mechanosensitive and is the site where contractions are initiated; (2) that hitting the stalk evokes a contraction because the hit exerts a mechanical effect on the cell body; and (3) that the rate of expansion of the membrane of the cell body is responsible for activation of a hypothetical mechanoreceptor mechanism which initiates a contraction.

Introduction

The peritrich ciliate *Vorticella* is known to exhibit a cellular contraction, involving shrinkage of the cell body and coiling of the stalk, in response to mechanical agitation (such as water current), vibration of the water and/or of the substratum, or collision with a solid object. Mechanosensitivity is a common characteristic among a variety of living cells, from bacteria to mechanoreceptor

* Present address and address for reprint requests: JSPS Liaison Office, Bonn, 'Bonn-Center', HI-1104, Bundeskanzlerplatz 2-10, 5300 Bonn 1, Germany.

Key words: mechanoreception, cellular contraction, *Vorticella*.

cells of vertebrates (Martinac *et al.* 1987, 1990; Gustin *et al.* 1988; Naitoh, 1982, 1984; Machemer, 1985; Hudspeth, 1989).

In many ciliate protozoans, the mechanosensitive Ca^{2+} channels responsible for producing an avoiding response are present predominantly in the anterior membrane, while mechanosensitive K^+ channels responsible for the escape response are present predominantly in the posterior membrane (Naitoh and Eckert, 1969; Naitoh, 1974). Mechanosensitive ion channels are also distributed in localized regions in vertebrate mechanoreceptor cells. They are restricted to the nerve ending of a Pacinian corpuscle (Loewenstein, 1971) and mechanoelectrical transduction channels are present around the tip (Hudspeth, 1979, 1982) or the base (Ohmori, 1988) of stereocilia of the hair cell.

The aspects of a mechanical stimulus effective in evoking a cellular response are different in different types of cells. Mechanoreceptor channels of patch-clamped chick skeletal muscle cells, frog oocytes, yeast cells and bacteria can be gated by hydrostatic pressure (Guharay and Sachs, 1985; Martinac *et al.* 1987; Taglietti and Toselli, 1988; Gustin *et al.* 1988). The magnitude of the mechanoelectrical transducer current of a voltage-clamped vertebrate hair cell is dependent on the absolute displacement of its stereocilium (Hudspeth, 1989). Both the rate and the extent of stretching of a tendon influence the activation of the tendon receptor of the cat (Ruffini ending of the knee-joint) (Boyd and Roberts, 1953). The rate of creation of a localized depression in the surface membrane affects the activation of mechanosensitive ion channels in many ciliate protozoans such as *Paramecium caudatum*, *Euplotes* sp., *Stylonychia lemnae* and *Tetrahymena pyriformis* (Naitoh *et al.* 1972; De Peyer and Machemer, 1978; Naitoh, 1984; Machemer, 1985). The great variation in the effective factor among different types of mechanoreceptor cells is attributable, at least in part, to differences in their viscoelastic properties.

Vorticella is an excellent model system for quantitative studies of mechanosensory transduction, because of the cell's high mechanosensitivity, its lack of mechanoreceptor specializations (which results in simpler mechanical properties, which are easier to analyse) and its large size.

The primary objectives of the research described in this paper were (1) to examine any localized difference in the mechanosensitivity for initiating a cellular contraction; (2) to investigate the possible involvement of ion channels in the mechanosensory mechanism; and (3) to determine the factor(s) of a mechanical stimulus that is effective in evoking a cellular contraction in *Vorticella*. The biological significance of the contraction is discussed in relation to the underlying factor(s). Some of these results have been presented verbally elsewhere (Katoh and Naitoh, 1988, 1991).

Materials and methods

Specimens of *Vorticella* sp. were grown at 20°C on a glass slide in a bacterized saline solution (final concentrations 0.1 mmol l^{-1} KCl, 0.09 mmol l^{-1} $CaCl_2$ and 0.1 mmol l^{-1} $MgSO_4$) made from dehydrated cereal leaves (Sigma) dissolved at

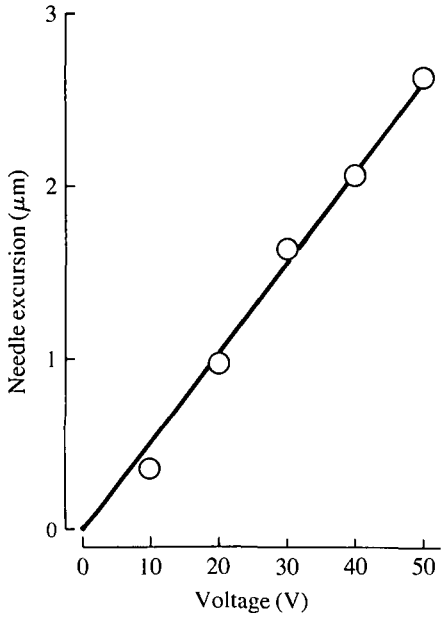

Fig. 1. The relationship between the excursion of the tip of a microneedle (ordinate) and the voltage of a square electric pulse applied to a phonocartridge on which the needle was mounted (abscissa). Each point is the mean±S.D. of five measurements. Error bars are omitted since they are smaller than the symbols. The line of best fit was drawn by eye.

0.1% (w/w) in water. Specimens of medium size (approximately 60 μm in cell body length) were washed with the 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 were kept immersed in the solution for about 10 min prior to experimentation.

The tip of a microneedle (10 μm in diameter) mounted on a piezoelectric phonocartridge was placed in gentle contact with the cell surface with the aid of a micromanipulator. The tip was driven perpendicular to the cell surface by activating the piezoelectric phonocartridge with an electric pulse.

The magnitude and rate of excursion of the tip were controlled by changing the voltage and the rate of change of the voltage of an electric current pulse applied to the cartridge. The excursion of the tip increased in proportion to the voltage of the pulse as shown in Fig. 1. The rate of excursion was calculated by dividing the degree of excursion by the time taken for the electric pulse to reach its final level. All the experiments were performed at room temperature (20–21°C). Further details of the experimental procedures will be described in the Results section.

Results

Cellular contractions caused by a mechanical stimulus

Specimens of *Vorticella* contracted when the tip of the microneedle was pressed

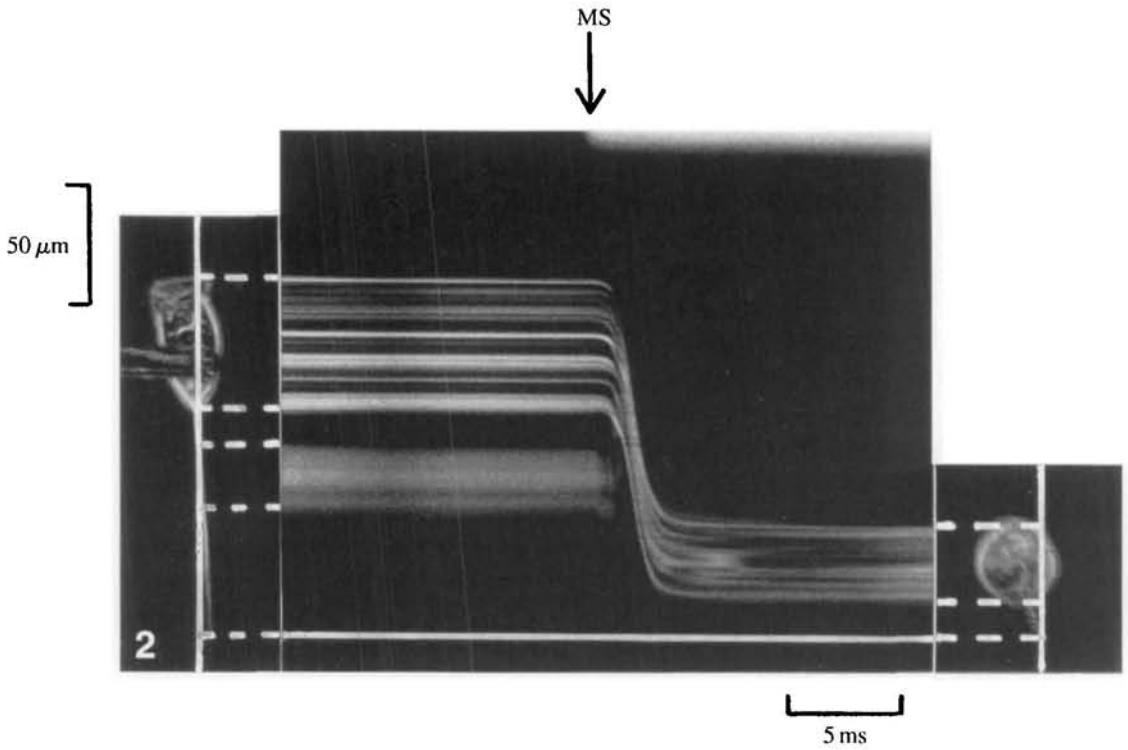

Fig. 2. Time course of a contraction (contraction curve) in a specimen of *Vorticella* evoked by a mechanical stimulus applied to the cell body. A contracting specimen was photographed on moving film using a slit camera. The picture on the left is of the specimen before contraction, and that on the right is during contraction. The vertical white line on each picture indicates the position of the slit relative to the image of the specimen. At the time labelled MS (mechanical stimulation) an electric pulse was applied to the phonocartridge, which then drove the microneedle against the cell body to evoke the contraction.

against them. Such a contraction is illustrated in Fig. 2, a photograph taken on moving film using a slit camera. Contractions were of an all-or-nothing type. The latency between a stimulus and the contraction was 1.7±0.2 ms (mean±S.D., N=10). The maximum rate of contraction (calculated from the slope of the contraction curve) was 6.7±2.1 cm s^{-1} (N=10). Contracted specimens slowly extended to their original length in 3.3±0.9 s (N=10).

Local differences in mechanosensitivity

To determine local differences in the mechanosensitivity which initiates contraction, the microneedle was pushed against various portions of a specimen attached to the substratum, and the threshold excursion required to elicit a contraction was determined in each portion. Four groups of specimens with stalks

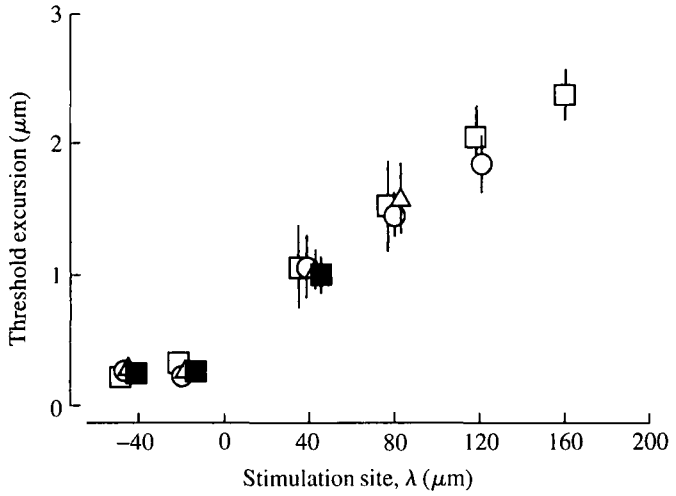

Fig. 3. Topographical difference in the mechanical threshold for evoking a contraction in specimens of *Vorticella*. Four different groups of specimens with stalks of different lengths were examined (filled squares, 80 μm; open triangles, 120 μm; open circles, 160 μm; open squares, 200 μm). The threshold, defined as the excursion of the microneedle just sufficient to evoke a contraction, is plotted against the stimulation site (λ), which is defined as the distance from the junction between the cell body and the stalk to the stimulation site. A positive value of λ is the distance measured towards the stalk and a negative value of λ is the distance measured towards the cell body. Values are mean±S.D., N=5. Note that symbols have been offset slightly for clarity.

of different lengths (80, 120, 160 and 200 μm), obtained from the same culture, were examined.

Fig. 3 shows the relationship between the threshold excursion of the microneedle and the site of stimulation, which was expressed as the distance from the junction between the cell body and the stalk to the stimulation site (λ). The threshold was lowest and identical at all sites on the cell body (negative λ region) (the threshold at −20 μm was not statistically different from that at −40 μm). On the stalk, it was higher when the site of stimulation was farther from the cell body (λ was longer) (the threshold at 160 μm was significantly higher than that at 40 μm; P<0.001). The relationships between threshold and λ were not statistically different among the four different groups of specimens, irrespective of their stalk length.

Mechanical clamping of the stalk influenced the effect of mechanical stimulation of the stalk

A specimen was first detached from its substratum (a glass slide) and laid on a second glass slide. The middle portion of the stalk was then mechanically clamped by pressing it against the glass slide with a microneedle. A mechanical stimulus (with a supramaximal excursion) was applied to the clamped stalk to examine whether it evoked a contraction.

When *Vorticella* was stimulated in a region between the clamped site and the cell body (proximal region), the whole cell contracted (the cell body shrank and the stalk coiled). In contrast, when *Vorticella* was stimulated in a region between the clamped site and the distal end of the stalk (distal region) there was no resulting contraction. When the clamp was removed, stimulating the same distal region again evoked a contraction of the whole cell.

Mechanosensitivity of specimens with the spasmoneme experimentally cut or naturally detached from the myoneme

When the middle portion of the stalk of a specimen (detached from its substratum and laid on a glass slide) was pressed very firmly against the glass slide with a microneedle, the portion was crushed flat and the spasmoneme was cut. A mechanical stimulus was applied to the stalk either proximal or distal to the point of crush after the microneedle had been removed from the crushed portion. The cell body shrank and the proximal region of the stalk coiled, while the distal region of the stalk did not.

The spasmoneme often became detached from the myoneme while the sheath of the stalk remained attached to the cell body and the specimen swam away as a free-swimming form, leaving its stalk behind. Mechanical stimuli applied to the stalk of spasmoneme-detached specimens evoked only shrinkage of the cell body. The stalk did not exhibit coiling. However, the mechanical threshold of the stalk was not statistically different from that of normal specimens (spasmoneme-detached specimens, $0.86\pm0.16\,\mu m$, $N=5$; normal, spasmoneme-attached specimens, $0.96\pm0.09\,\mu m$, $N=5$; $\lambda=40\,\mu m$).

It should be noted here that pressing the stalk against the glass slide in order to cut the spasmoneme did not evoke a contraction if care was taken not to agitate the cell body mechanically. Furthermore, drawing a small portion of the stalk into a glass pipette (approximately $7\,\mu m$ inner diameter) by suction did not evoke a contraction, whereas drawing any portion of the cell body into the capillary in a similar way did.

Effects of external cations on the mechanosensitivity

To examine whether ion channel activity in the surface membrane was involved in *Vorticella*'s mechanoreception, the relationships between stimulus threshold and λ were determined in solutions with different cationic compositions. As shown in Fig. 4, the relationship was not affected by raising the K^+ concentration to 20 mmol l^{-1} or raising the Ca^{2+} concentration to 10 mmol l^{-1}. The relationship remained the same after Ca^{2+} had been removed from the external solution by addition of EGTA (5 mmol l^{-1} final concentration).

The relationship between the threshold degree of tilting of the stalk and the rate of tilting needed to evoke a cellular contraction

Pressing a microneedle against a specimen attached to the substratum by its stalk caused translocation and rotation of the cell as well as depression of the cell

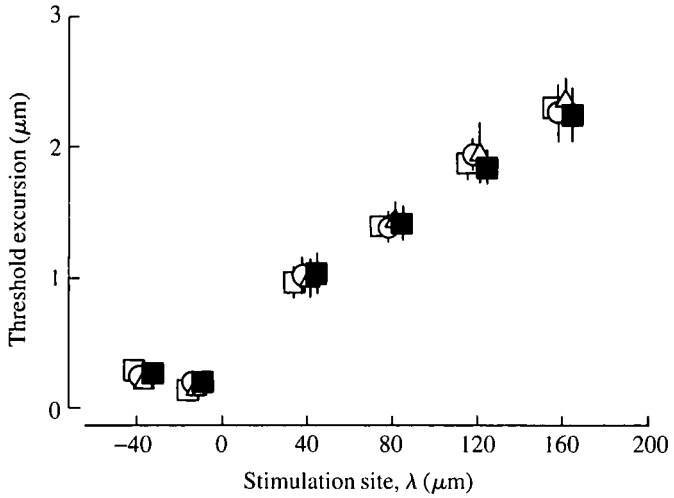

Fig. 4. The relationship between threshold excursion and stimulation site (λ) obtained in specimens of *Vorticella* immersed in solutions with different ionic compositions. Open squares, 1 mmol l^{-1} KCl+1 mmol l^{-1} $CaCl_2$ (control); open circles, 20 mmol l^{-1} KCl+1 mmol l^{-1} $CaCl_2$; open triangles, 1 mmol l^{-1} KCl+10 mmol l^{-1} $CaCl_2$; filled squares, 1 mmol l^{-1} KCl+5 mmol l^{-1} potassium EGTA. See the legend of Fig. 3 for the definition of threshold and λ. The values are mean±S.D., N=5. Note that symbols have been offset slightly for clarity.

surface at the point of contact or a bending of the stalk. To eliminate the translocation and rotation, specimens were detached from their substratum and their cell body was fixed at the tip of a micropipette (3 μm internal diameter) by lowering the hydrostatic pressure inside the pipette. In these specimens, stimulating the cell body mechanically produced only a depression in the surface membrane, while stimulating the free stalk caused it to tilt relative to the cell body. Tilting the stalk evoked a cellular contraction similar to that caused by stimulating the cell body.

The excursion of the microneedle required to tilt the stalk just far enough to evoke a contraction (the threshold excursion) was determined while the position of the tip of the microneedle on the stalk was varied. The rate of excursion of the microneedle was adjusted so that the stalk tilted at a constant angular velocity, ω, irrespective of the position of the tip. The threshold excursion was plotted against the position of the tip on the stalk, which was expressed as the distance from the junction between the cell body and the stalk to the tip. Fig. 5 shows two sets of plots obtained in two series of experiments with different values of ω (17.5 rad ms^{-1} and 30.7 rad ms^{-1}). The threshold excursion increased in proportion to the distance, and it was significantly higher when ω was lower (P<0.01 for the thresholds at 30 and 40 μm). The slope of the plot corresponds to the threshold degree of tilting, θ_t, which is expressed as the angle between the stalk

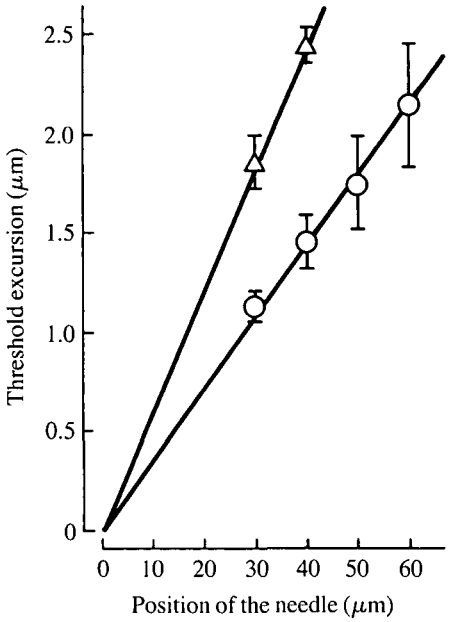

Fig. 5. The relationship between the threshold excursion of a microneedle required to evoke a contraction (ordinate) and the position of the needle on the stalk (abscissa) in specimens of *Vorticella* isolated from their substratum. The position of the needle is expressed as the distance from the junction between the cell body and the stalk to the needle. The angular velocity of tilting of the stalk (ω) was 30.7 rad ms^{-1} (triangles) and 17.5 rad ms^{-1} (circles). The values are mean±S.D., N=5. The lines of best fit were drawn by eye.

and the longitudinal axis of the cell body. θ_t was lower when ω was higher (0.036 rad when ω was 30.7 rad ms^{-1} and 0.062 rad when ω was 17.5 rad ms^{-1}).

In the next series of experiments, the relationship between θ_t and ω was examined more precisely. Fig. 6 shows a plot of θ_t against ω, both on logarithmic axes. The relationship was linear with a slope of -1. This indicates that θ_t is inversely proportional to ω. The proportionality constant was calculated from the intersection of the plot with the threshold axis (ordinate) to be 1.09 $rad^2\,ms^{-1}$.

The relationship between the degree of depression of the cell body and the rate of depression required to evoke a cellular contraction

In this series of experiments, the microneedle was pushed against the middle portion of the cell body of a detached and fixed specimen, and the degree of depression just sufficient to evoke a contraction (the threshold depression), x_t, was determined while the rate of depression, dx/dt, was varied.

Fig. 7 shows a plot of x_t against dx/dt, both on logarithmic axes. The plot was linear with a slope of -1 for values of dx/dt in the range 8–40 $\mu m\,ms^{-1}$. This indicates that x_t is inversely proportional to dx/dt in this range. The proportion-

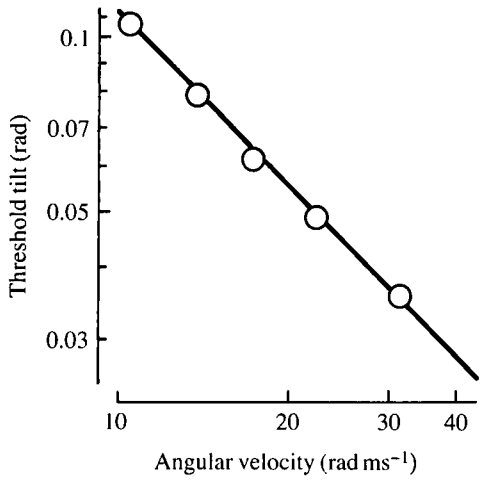

Fig. 6. The relationship between the threshold tilt of the stalk required to evoke a contraction (ordinate) and the angular velocity of tilting (abscissa) in specimens of *Vorticella* isolated from their substratum. Tilt is expressed as the angle between the longitudinal axes of the stalk and the cell body. The threshold tilt at the angular velocity of 17.5 rad ms^{-1} and that at 30.7 rad ms^{-1} were derived from the data presented in Fig. 5. Values are mean±S.D., N=5. Error bars are omitted since they are smaller than the symbols. The line of best fit was drawn by eye.

ality constant was calculated from the intersection of the plot with the threshold axis (ordinate) to be 11.8 μm^2 ms^{-1}.

The deviation from linearity at the lowest value of dx/dt (4 μm ms^{-1}) (lower than the value expected from the line) is attributable to the limitation of the excursion of the microneedle. As shown in Fig. 1, the maximum excursion of the microneedle is 2.7 μm (at 50 V). This value is close to the threshold depression at 4 μm ms^{-1}. Some specimens, therefore, contracted in response to the depression (and some responded to a lesser depression) while others did not respond. Thus, the mean threshold value for the responding specimens was lower than the true value. The deviation from linearity at the highest dx/dt (50 μm ms^{-1}) (higher than the value expected from the line) might be attributable to the limitation of the rapid movement of the microneedle (due to the overall mass of the phonocartridge and the microneedle).

Discussion

Pushing of the tip of a microneedle against the stalk of *Vorticella* evoked a contraction. The threshold excursion of the microneedle required was larger in regions farther from the cell body and smaller in the cell body itself (Figs 3, 4). However, stimulating the stalk did not evoke a contraction when the stalk was kept mechanically clamped in a region between the site of stimulation and the cell body. Moreover, pressing the stalk against a glass slide using a microneedle, or

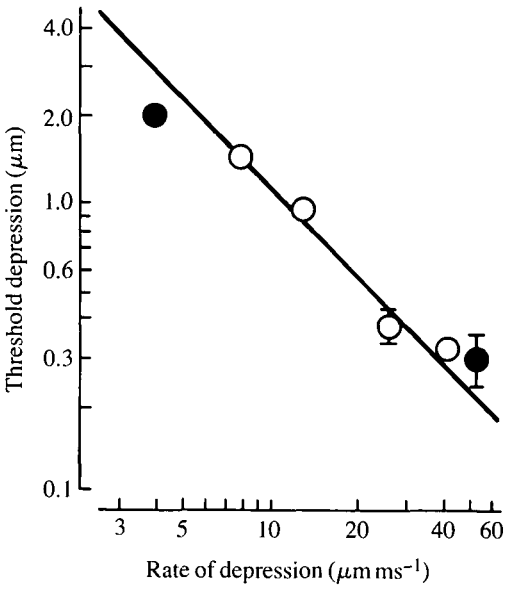

Fig. 7. The relationship between the threshold degree of depression of the cell body (ordinate) and the rate of depression (abscissa) required to evoke a contraction in specimens of *Vorticella* isolated from their substratum. The tip of a microneedle was pressed against the cell body to produce a depression in the cell surface. Values are mean±S.D., $N=5$. The line of best fit was drawn by eye for the four points with rates of depression in the range 8–40 μm ms^{-1} (open circles); the two points at the extremes (4 and 50 μm ms^{-1}; filled circles) were ignored. See the text for the details.

quickly sucking a portion of the stalk into a micropipette, did not evoke a contraction, whereas similar suction of a portion of the cell body always evoked a contraction. These observations imply that the cell body has a mechanoreceptor mechanism for evoking a contraction, while the stalk has not. We therefore presume that stimulating the stalk exerts a mechanical effect on the mechanosensitive cell body, thus evoking a cellular contraction, and that the higher mechanical threshold in regions of the stalk farther from the cell body is attributable to a decrement of the mechanical effect according to the distance from the site of stimulation to the cell body. Machemer-Röhnisch and Machemer (1984) reported a similar decrement in the efficiency with which mechanical stimuli applied to the caudal cilia of *Paramecium* could activate mechanosensitive ion channels in the posterior membrane.

Stimulating a stalk with an experimentally cut spasmoneme evoked a contraction. This implies that continuity of the spasmoneme in the stalk is not necessary for the transfer of the mechanical effect to the cell body. The sheath of the stalk seems to be responsible for the transfer.

Activation of the myoneme to shrink the cell body and activation of the spasmoneme to coil the stalk are dependent on Ca^{2+} (Hoffmann-Berling, 1958;

Amos, 1972*a*, 1975; Amos *et al.* 1975, 1976; Ochiai *et al.* 1983). We found that the mechanical threshold for evoking a contraction was not affected by removal of Ca^{2+} from the external solution (Fig. 4). This is consistent with the finding of Allen (1973) that *Vorticella* could contract even in a Ca^{2+}-deficient solution (see also Shiono and Naitoh, 1978). Ca^{2+}, therefore, must be liberated from some intracellular storage sites in response to a mechanical stimulus (Carasso and Favard, 1966; Amos, 1972*b*). Some previous workers (Moreton and Amos, 1979; Shiono *et al.* 1980) proposed that an influx of Ca^{2+}, accompanied by a Ca^{2+} spike generated in response to a mechanical stimulus, is responsible for evoking contraction in vorticellid protozoans. However, we found that the mechanical threshold for evoking a cellular contraction was largely unaffected by a large increase in external K^+ concentration (Fig. 4), which brought about a large depolarization of the membrane and thus diminished the Ca^{2+} spike (Shiono and Naitoh, 1978). It is therefore highly probable that activation of Ca^{2+} channels in the surface membrane is not directly involved in the mechanoreception in *Vorticella* that evokes a contraction.

The stalks of specimens with experimentally cut spasmonemes coiled only in the region between the cell body and the cut portion. Moreover, coiling of the stalk was not observed in specimens in which the spasmoneme had been detached from the myoneme at the junction between the cell body and the stalk. These observations indicate that the connection between the spasmoneme and the myoneme is required for initiation of coiling and that continuity of the spasmoneme in the stalk is needed for propagation of coiling along the stalk.

Sugi (1959, 1960) found that coiling of the stalk of *Carchesium* sp., a relative of *Vorticella*, started at the junction between the cell body and the stalk and propagated down the stalk. The coiling propagated beyond a mechanically clamped region (see also Jones *et al.* 1970). We confirmed these findings. This suggests that an increase in Ca^{2+} concentration around the spasmoneme occurs first at the myoneme–spasmoneme junction, then spreads down the spasmoneme. We found that the stalk itself was mechanically insensitive. It is, therefore, not feasible that the sequential liberation of Ca^{2+} from storage sites, such as membrane-bound tubules along the spasmoneme (Carasso and Favard, 1966; Amos, 1972*b*; Allen, 1973), is activated by their consecutive mechanical stretching. Instead Ca^{2+}-induced Ca^{2+} release from the sites (Endo *et al.* 1970) is the most probable mechanism by which Ca^{2+} release is propagated along the spasmoneme. It should be noted that the initiation of a contraction of *Vorticella* by injection of an inward current requires the presence of external Ca^{2+} (Shiono and Naitoh, 1978). Moreover, Jones *et al.* (1970) reported that coiling initiated in the stalk by injection of electric current propagated towards both the distal and the proximal ends of the stalk. Ca^{2+} driven into the cell body by the current might induce a release of Ca^{2+} from Ca^{2+} storage sites.

Our quantitative examination of detached specimens and those with fixed cell bodies revealed that the threshold degree of tilting of the stalk required to evoke a contraction (θ_t) was inversely proportional to ω (Fig. 6). This means that the

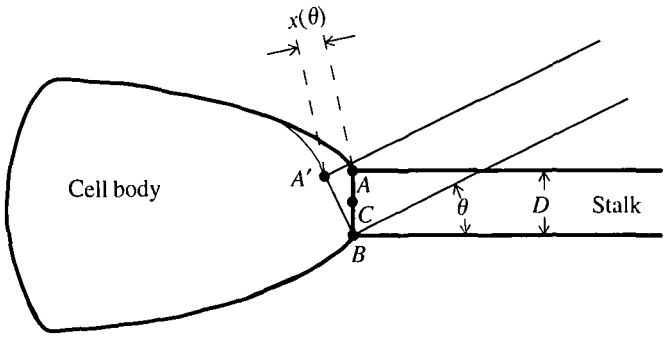

Fig. 8. A diagram of a sagittal section of a specimen of *Vorticella* which allows estimation of the degree of a localized depression of the surface membrane of the cell body caused by tilting the stalk. The scheme shows only the case when the stalk tilts around one of its edges (*B*). The opposite edge (*A*) moves to a position labelled *A'* to make a depression, $x(\theta)$, in the surface membrane. It is presumed that the depression of the membrane produces a membrane expansion, which activates the mechanosensory mechanism. θ, the degree of tilting. *D*, the thickness of the stalk. *C* is a point midway between *A* and *B*.

product $\theta_t\omega$ is a constant. In other words, a contraction occurs whenever the product exceeds a certain threshold value (the threshold product), irrespective of the value of ω. The threshold product corresponds to the proportionality constant ($1.09\,\mathrm{rad^2\,ms^{-1}}$).

It is presumed that tilting of the stalk causes a localized depression and/or elevation of the surface membrane around the junction between the cell body and the stalk, which activates a hypothetical mechanoreceptor mechanism in the membrane and evokes a contraction. As shown schematically in Fig. 8, one edge of the stalk (*A*) produces a depression in the surface membrane when the stalk pivots around the opposite edge (*B*). When the stalk pivots around a point between *A* and *B* (such as *C*), edge *A* produces a depression, while edge *B* produces an elevation. When the stalk pivots around *A*, edge *B* produces an elevation. For simplicity, we will consider here only the case where the stalk pivots around *B*. The size of the depression $x(\theta)$ caused by a tilt (θ rad) can be expressed as:

$$x(\theta) = D\theta, \tag{1}$$

where *D* is the thickness of the stalk. The rate of depression, $\mathrm{d}x(\theta)/\mathrm{d}t$ can be expressed as:

$$\mathrm{d}x(\theta)/\mathrm{d}t = D\omega, \tag{2}$$

where ω is the angular velocity of tilting and *t* is time. The product of the threshold extent of depression and the corresponding rate of depression, $x(\theta)_t\mathrm{d}x(\theta)/\mathrm{d}t$ can be written as:

$$x(\theta)_t\mathrm{d}x(\theta)/\mathrm{d}t = D^2\theta_t\omega, \tag{3}$$

which is a constant. Equation 3 implies that a contraction occurs when this product

is above a certain threshold value. The threshold value can be estimated by using a value of 3.4 μm for D (a mean of six measurements) and 1.09 $rad^2\,ms^{-1}$ (from Fig. 6) for $\theta_t\omega$ in equation 3. Using these values, the threshold is 12.7 $\mu m^2\,ms^{-1}$.

We also examined the relationship between the threshold extent of a localized depression of the surface membrane caused by pushing the surface and the rate of depression required to evoke a contraction. As shown in Fig. 7, the threshold extent of depression, x_t, was inversely proportional to the rate of depression, dx/dt. This means that a contraction occurs when the product xdx/dt is above a certain threshold value (the threshold product; $x_t dx/dt$). This threshold is estimated to be 11.8 $\mu m^2\,ms^{-1}$, similar to that for $x(\theta)_t dx(\theta)/dt$ (estimated from the threshold product $\theta_t\omega$), which is the threshold for evoking a contraction by tilting the stalk (12.7 $\mu m^2\,ms^{-1}$). This strongly supports the hypothesis that tilting causes a localized depression of the cell membrane, thus producing a contraction.

It is interesting to note that the threshold product $x_t dx/dt$ [and also $x(\theta)_t dx(\theta)/dt$] has the dimensions of L^2T^{-1} (L, length; T, time), which corresponds to that for the rate of change in the area. Pushing a microneedle against the cell surface stretches the inner membrane (and thus the endoplasmic reticulum) as well as the surface membrane, since the endoplasmic reticulum is present immediately beneath the surface membrane (Allen, 1973). It is, therefore, highly probable that the rate of expansion of the membrane is a major factor activating the hypothetical mechanosensory system responsible for evoking a contraction in *Vorticella*. The mechanism by which the rate of membrane expansion is detected remains unclear.

It is presumed that a contraction of *Vorticella* is a behavioural response which allows it to escape from a dangerous stimulus. Mechanical stimuli which produce more rapid expansion of the membrane may be more dangerous to *Vorticella*, because they might damage the cell. Indeed, *Vorticella* always contracted when subjected to a fast water current, whereas it rarely contracted even when its cell body was tilted at a sharp angle against the stalk by the pressure of a slow water current. The fast water current produces more rapid tilting of the cell body against the stalk, and this apparently causes a membrane expansion that is fast enough to activate the contractile mechanism. In contrast, the slow tilting of the cell body produced by a slow water current causes a membrane expansion that is too slow to activate the contractile mechanism.

This work was supported by a grant in Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan and a grant from the Mitsubishi Foundation to Y.N. We are grateful to Professor D. R. J. Macer for critically reading the manuscript and to Dr H. Horigami of Hosei University for giving us specimens of *Vorticella*.

References

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

AMOS, W. B. (1972*a*). Evidence for a new mechanism of cell motility. *Nature* **236**, 301–304.
AMOS, W. B. (1972*b*). Structure and coiling of the stalk in the peritrich ciliates *Vorticella* and *Carchesium*. *J. Cell Sci.* **10**, 95–122.
AMOS, W. B. (1975). Contraction and calcium binding in the vorticellid ciliates. In *Molecules and Cell Movement* (ed. S. Inoue and R. E. Stephens), pp. 411–436. New York: Raven Press.
AMOS, W. B., ROUTLEDGE, L. M., WEIS-FOGH, T. AND YEW, F. F. (1976). The spasmoneme and calcium-dependent contraction in connection with specific calcium binding proteins. *Symp. Soc. exp. Biol.* **30**, 273–301.
AMOS, W. B., ROUTLEDGE, L. M. AND YEW, F. F. (1975). Calcium-binding proteins in a vorticellid contractile organelle. *J. Cell Sci.* **19**, 203–213.
BOYD, I. A. AND ROBERTS, T. D. M. (1953). Proprioceptive discharges from stretch-receptors in the knee-joint of the cat. *J. Physiol., Lond.* **122**, 38–58.
CARASSO, N. AND FAVARD, P. (1966). Mise en évidence du calcium dans les myonèmes pédonculaires de ciliés péritriches. *J. Microscopie* **5**, 759–770.
DE PEYER, J. E. AND MACHEMER, H. (1978). Hyperpolarizing and depolarizing mechanoreceptor potentials in *Stylonychia*. *J. comp. Physiol.* **127**, 255–266.
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.
GUHARAY, F. AND SACHS, F. (1985). Stretch-activated single ion channel currents in tissue-cultured embryonic chick skeletal muscle. *J. Physiol., Lond.* **352**, 685–701.
GUSTIN, M. C., ZHOU, X. L., MARTINAC, B. AND KUNG, C. (1988). A mechanosensitive ion channel in the yeast plasma membrane. *Science* **242**, 762–764.
HOFFMANN-BERLING, H. (1958). Der Mechanismus eines neuen, von der Muskelkontraktion verschiedenen Kontraktionszyklus. *Biochim. biophys. Acta* **27**, 247–255.
HUDSPETH, A. J. (1979). Stereocilia mediate transduction in vertebrate hair cells. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1506–1509.
HUDSPETH, A. J. (1982). Extracellular current flow and the site of transduction by vertebrate hair cells. *J. Neurosci.* **2**, 1–10.
HUDSPETH, A. J. (1989). How the ear's works work. *Nature* **341**, 397–404.
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 NAITOH, Y. (1988). Contraction of *Vorticella* in response to a mechanical stimulus. I. Localization of mechanoreception mechanism. *Zool. Sci.* **5**, 1217.
KATOH, K. AND NAITOH, Y. (1991). Mechanoreception and a cellular contraction in a vorticellid protozoan. *Comp. Physiol. Biochem.* (in press).
LOEWENSTEIN, W. R. (1971). Mechano-electric transduction in the Pacinian corpuscle. Initiation of sensory impulses in mechanoreceptors. In *Handbook of Sensory Physiology*. 1. *Principles of Receptor Physiology* (ed. W. R. Loewenstein), pp. 269–290. Berlin: Springer-Verlag.
MACHEMER, H. (1985). Mechanoresponses in Protozoa. In *Sensory Perception and Transduction in Aneural Organisms* (ed. P. S. Song, G. Colombetti and F. Lenci), pp. 179–209. New York: Plenum Press.
MACHEMER-RÖHNISCH, S. AND MACHEMER, H. (1984). Receptor current following controlled stimulation of immobile tail cilia in *Paramecium caudatum*. *J. comp. Physiol.* A **154**, 263–271.
MARTINAC, B., ADLER, J. AND KUNG, C. (1990). Mechanosensitive ion channels of *E. coli* activated by amphipath. *Nature* **348**, 261–263.
MARTINAC, B., BUECHNER, M., DELCOUR, A. H., ADLER, J. AND KUNG, C. (1987). A pressure-sensitive ion channel in *Escherichia coli*. *Proc. natn. Acad. Sci. U.S.A.* **84**, 2297–2301.
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. (1974). Bioelectric basis of behavior in protozoa. *Am. Zool.* **14**, 883–895.
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. (1984). Mechanosensory transduction in Protozoa. In *Membranes and Sensory Transduction* (ed. G. Colombetti and F. Lenci), pp. 113–135. New York: Plenum Press.
NAITOH, Y. AND ECKERT, R. (1969). Ionic mechanisms controlling behavioral responses of *Paramecium* to mechanical stimulation. *Science* **164**, 963–965.
NAITOH, Y., ECKERT, R. AND FRIEDMAN, K. (1972). Sensory mechanisms in *Paramecium*. 1. Two

components of the electric response to mechanical stimulation of the anterior surface. *J. exp. Biol.* **56**, 683–694.

Ochiai, T., Hara, R. and Asai, H. (1983). Contraction of the spasmoneme and coiling of the sheath in the glycerinated stalk of *Vorticella*. *Cytobios* **36**, 95–105.

Ohmori, H. (1988). Mechanical stimulation and Fura-2 fluorescence in the hair bundle of dissociated hair cells of the chick. *J. Physiol., Lond.* **399**, 115–137.

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. (1978). Membrane potential change associated with a contraction of the cell body in *Vorticella convallaria*. *Zool. Mag.* **87**, 429.

Sugi, H. (1959). Contraction and relaxation in the stalk muscle of *Carchesium*. *Annot. Zool. Japon.* **32**, 163–169.

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

Taglietti, V. and Toselli, M. (1988). A study of stretch-activated channels in the membrane of frog oocytes: Interaction with Ca^{2+} ions. *J. Physiol., Lond.* **407**, 311–328.