J. Exp. Biol. (1973), **59**, 53–65
With 13 text-figures
Printed in Great Britain

SENSORY MECHANISMS IN *PARAMECIUM*

II. IONIC BASIS OF THE HYPERPOLARIZING MECHANORECEPTOR POTENTIAL

BY YUTAKA NAITOH AND ROGER ECKERT

Department of Biology and Brain Research Institute, University of California, Los Angeles, California 90024

(*Received* 4 *December* 1972)

INTRODUCTION

Mechanical stimulation of the surface of a ciliate produces changes in membrane potential known to regulate ciliary activity (Naitoh & Eckert, 1969*a*, *b*; Eckert, 1972; Eckert & Naitoh, 1972). Stimulation of the caudal (posterior) surface evokes a transient hyperpolarization, while a similar stimulus to the anterior surface evokes a depolarization. The surface membrane is thus differentiated functionally at the two ends of the cell to produce potentials of opposite electrical sign in response to mechanical stimuli. The adaptive significance of the two differentiated receptor areas in the cell membrane is evident. When the ciliate collides head-on with an obstacle, the depolarization produced by the anterior receptor current leads to a reversal of the direction of the power stroke of its cilia, causing it to swim in reverse. This initiates the 'avoiding reaction' (Jennings, 1906). In contrast, the hyperpolarization evoked by a stimulus to the rear leads to an increase in beating frequency which results in the acceleration of forward swimming. This increase in the frequency of 'normal' forward-swimming beating always accompanies hyperpolarization (Naitoh, 1958; Kinosita, Dryl & Naitoh, 1964). Stimuli to the anterior and posterior ends of a ciliate thus evoke modifications of ciliary activity which in both cases tend to carry the ciliate away from the source of stimulation.

Evidence is presented in an earlier paper (Eckert, Naitoh & Friedman, 1972) that the primary depolarization in response to mechanical stimulation of the anterior end is due to an inward flow of receptor current. We now present evidence that mechanical stimulation of the caudal surface produces an outward current carried by potassium ions, presumably due to a transient local increase in K^+ conductance of the membrane. The outward receptor current causes a hyperpolarizing shift in membrane potential toward the potassium equilibrium potential. This potential transient is termed the *caudal receptor potential*. Other examples among the protozoa of receptor potentials produced in response to mechanical stimuli are found in the dinoflagellate *Noctiluca* (Eckert, 1965), the hypotrich ciliate *Euplotes* (Naitoh & Eckert, 1969*b*) and the heterotrich ciliate *Stentor* (Wood, 1970).

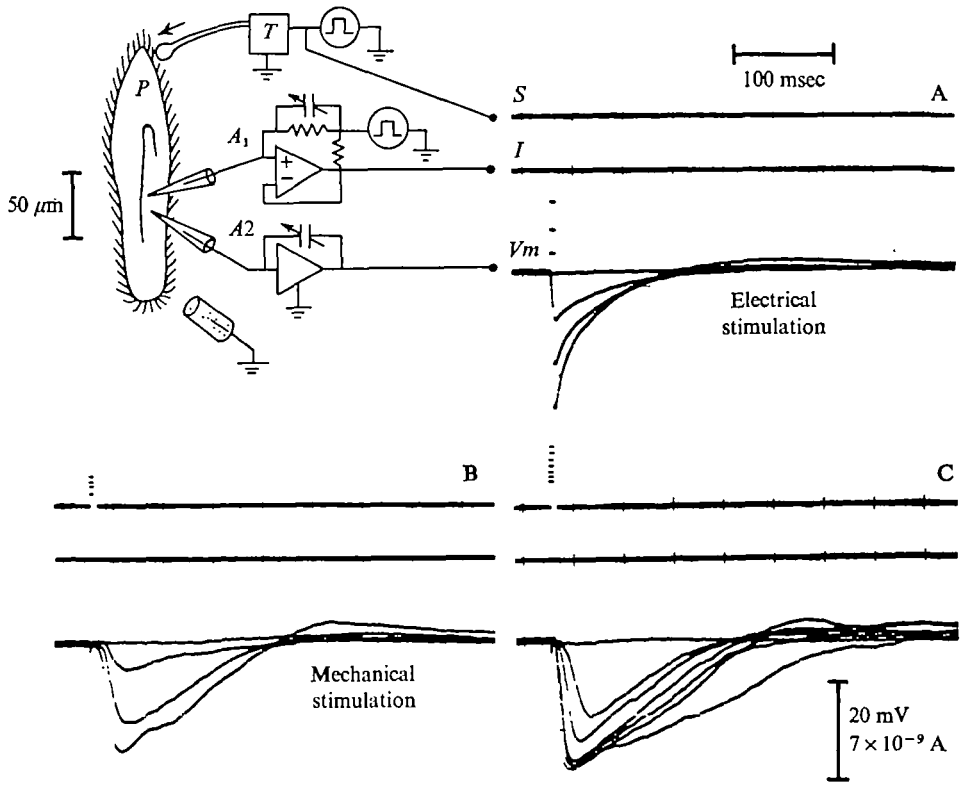

Fig. 1. Electrical and mechanical stimulation of *P. caudatum*. Upper left, Arrangement for stimulation and recording. Current and voltage electrodes were inserted into the central region. The posterior end (*P*) of the specimen was mechanically stimulated with a microstylus (10–30 μm in tip diameter) when a 5 msec voltage pulse was applied to piezoelectric transducer (*T*). *A* 1, constant-current pulse generator with capacitance compensation. *A* 2, head stage for intracellular recording. A, Graded electrical responses (lower trace, *Vm*) to hyperpolarizing electric pulses of 5 msec duration. Middle trace (*I*) shows current intensity. B and C, graded electrical responses (receptor potentials; lower traces, *Vm*) to mechanical stimulation. Upper trace (*S*), voltage pulses applied to transducer.

METHODS

Specimens of *Paramecium caudatum* cultured in a hay infusion were washed, equilibrated, isolated and secured under a microscope for experimentation as described elsewhere (Naitoh & Eckert, 1972). Methods of intracellular recordings, injection of current and application of mechanical stimuli were essentially the same as those described in an earlier paper (Eckert *et al.* 1972), except that mechanical stimuli were applied to the caudal region of *Paramecium*. Electric current was injected from a microelectrode into the cell by use of a constant-current generator which had a positive capacitance feed-back system (New, 1972) to minimize the distortion of the shape of pulse due to stray capacitance of the microelectrode (Fig. 1). All the experiments were performed at room temperatures of 17–21 °C.

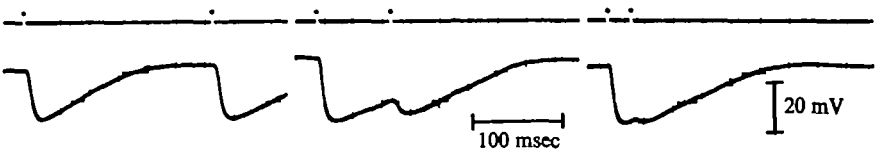

Fig. 2. Test for refractoriness and summation. Two mechanical stimuli of the same intensity were applied successively with different time intervals. Upper trace shows voltage pulses applied to piezoelectric transducer. Lower trace shows corresponding receptor potentials.

RESULTS

Electrical response to mechanical stimulation of the caudal surface

The response to mechanical stimulation of the caudal membrane was a rapid hyperpolarization which reached a 'peak' and then slowly returned to the resting level (Fig. 1 B, C). As the intensity of the mechanical stimulus was increased, the receptor potential showed a graded increase in amplitude up to a maximum. Further increase in the intensity resulted in a prolongation of the receptor potential. A larger receptor potential, caused by stronger stimuli, was always followed by a small depolarization before returning to the resting level. The time to reach the peak level became shorter with strong stimuli.

This receptor potential was compared with the response of the membrane to injected inward (hyperpolarizing) current of 5 msec duration (Fig. 1 A). The electrotonic response consisted of a rapid charging of the membrane capacitance during the pulse followed by a slower discharge, which was significantly more rapid than the recovery phase of the receptor potential after the peak. This response to injected current is consistent with the passive (i.e. electrotonic) electrical characteristics of *Paramecium* noted earlier (Eckert & Naitoh, 1970). Strong hyperpolarizations were followed by small depolarizations similar to that following large receptor potentials. These may be due to a small transient calcium activation as the membrane approaches the resting potential following a hyperpolarization.

The receptor potential exhibited no refractoriness. Neither did maximal receptor potentials show summation (Fig. 2). The caudal surface was more sensitive to mechanical stimuli than the anterior surface, requiring less displacement of the stylus for a maximal response. Hyperpolarizing responses could be elicited by movements of the stylus in the medium up to 50 μm away from the caudal surface.

The input resistance of the cell was monitored during the receptor potential as shown in Fig. 3 A. A series of bipolar square pulses from a constant-current generator were injected into the cell so as to produce RC potential changes superimposed on the receptor potential. The input resistance was determined from the slope of logarithmic plots of the time derivatives of the RC potentials against time. As shown in Fig. 4, an increase of conductance to more than six times its resting value was observed in the early phase of the receptor potential. The conductance then gradually returned to its pre-stimulus value as the receptor potential subsided.

The input resistance was measured during a simulated receptor potential produced by the injection of depolarizing current with a microelectrode (Fig. 3 B). A delayed increase in the conductance of up to twice the resting value was observed, as shown in Fig. 4. This was consistent with the previous finding of the delayed hyper-

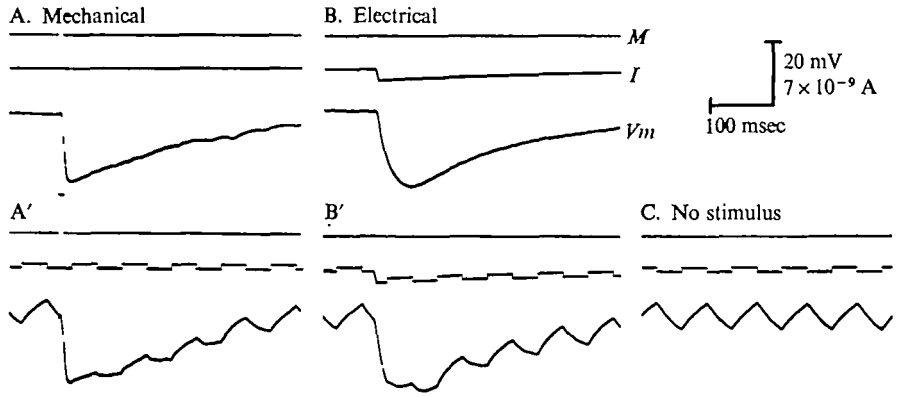

Fig. 3. Conductance change during mechanically evoked receptor potential (A) and during similar hyperpolarization simulated by electric current (B) injected with microelectrode. A train of bipolar pulses (40 msec) was injected to superimpose RC potentials on the electrical responses. Responses to mechanical and electrical stimuli without pulse train are shown in A and B respectively and with pulse train in A′ and B′. C shows the RC responses of the unstimulated membrane. Upper trace (*M*), voltage applied to transducer. Middle trace (*I*), current injected into the cell. Lower trace (*Vm*), membrane potential.

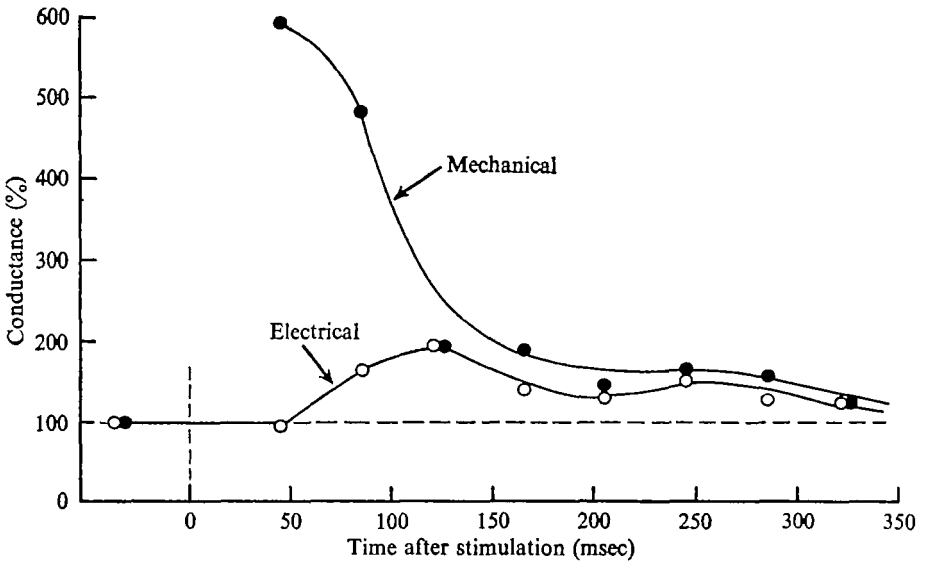

Fig. 4. Conductance change during mechanically evoked receptor potential and simulated hyperpolarization by electric current. Relative conductance was calculated from changes in the time constants of the RC potentials superimposed on the membrane responses. Solid circles come from A′ (receptor potential) and open circles from B′ (simulated hyperpolarization) respectively in Fig. 3.

polarizing rectification in *Paramecium* (Naitoh & Eckert, 1968). Mechanical stimulation therefore appears to produce a transient increase in membrane conductance. The conductance increase is not regenerative and is therefore believed to be limited to the region of membrane actually deformed by the stimulus.

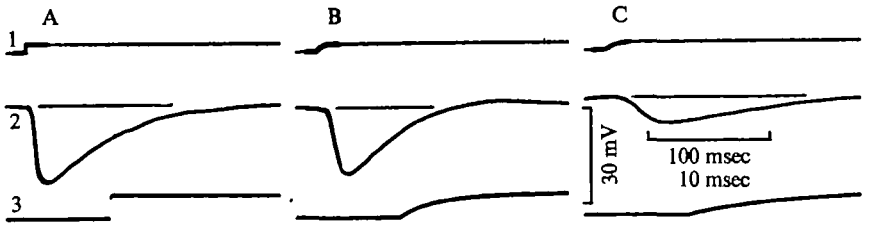

Fig. 5. Rate-sensitivity of the response. Trace 1, voltage applied to piezoelectric transducer. Trace 2, membrane potential. Trace 3, expanded sweep (10 times) of the intensified segment of trace 1. The time constant of voltage rise applied to the piezoelectric unit was increased from A to C, although the final voltage remained the same.

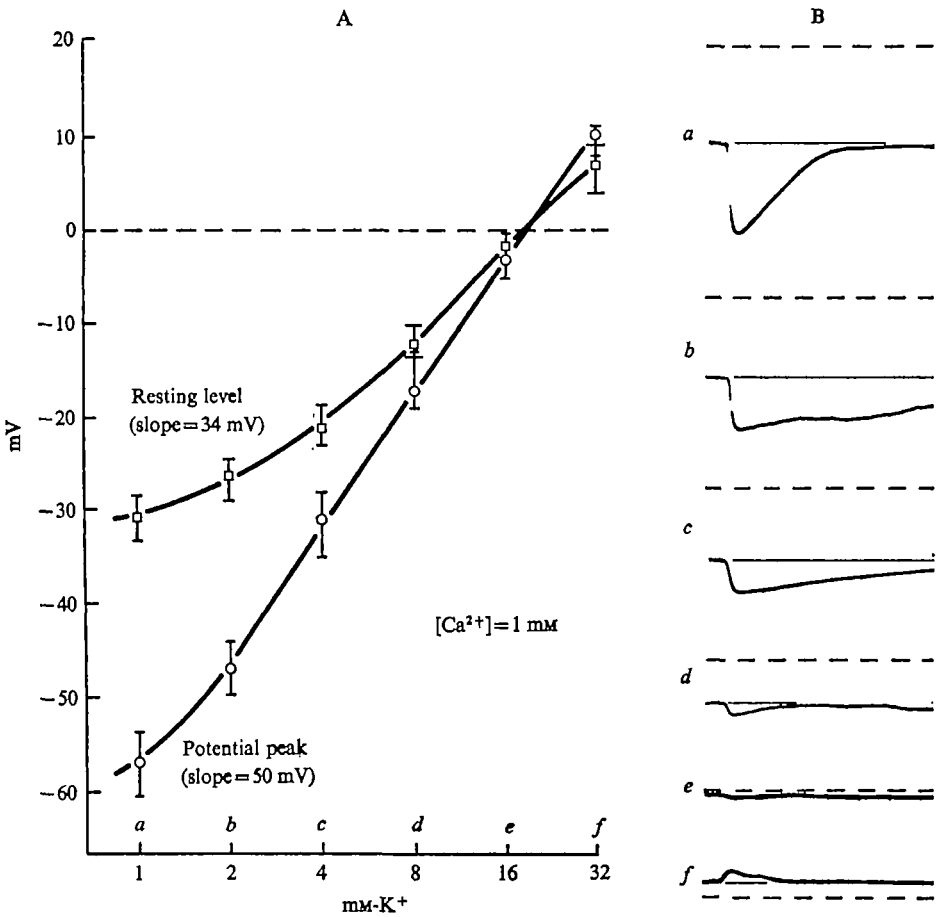

Fig. 6. Potential as a function of extracellular potassium. Calcium held constant throughout at 1 mM. A, resting potential and peak value of receptor potential plotted against log $(K)_o$. Resting potential dropped with a slope of 34 mV per 10-fold increase in $(K)_o$; receptor potential peak dropped with a slope of 50 mV. B, representative electrical records obtained at concentrations *a–f* in A. Dashed lines indicate reference (zero) potential for each recording. Each point is the mean of 3–6 measurements, with spread indicated.

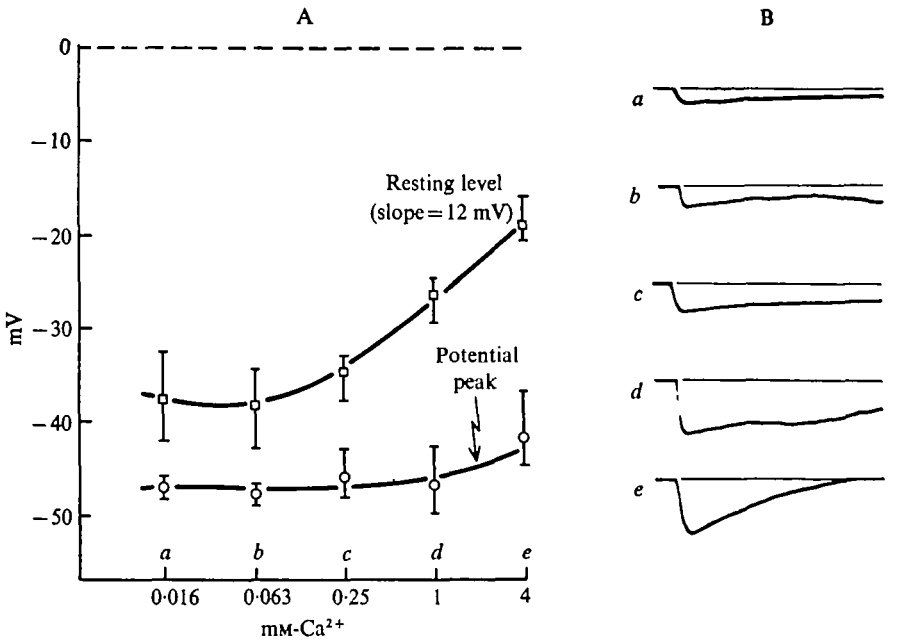

Fig. 7. Potential as a function of extracellular calcium. Potassium held constant throughout at 2 mM. A, resting potential and peak value of receptor potential plotted against log $(Ca)_0$. Resting potential shows a slope of 12 mV per 10-fold increase in $(Ca)_0$. B, representative records obtained at concentrations *a–e* in A. Each point on the graph is the mean of 3–6 measurements, with spread indicated.

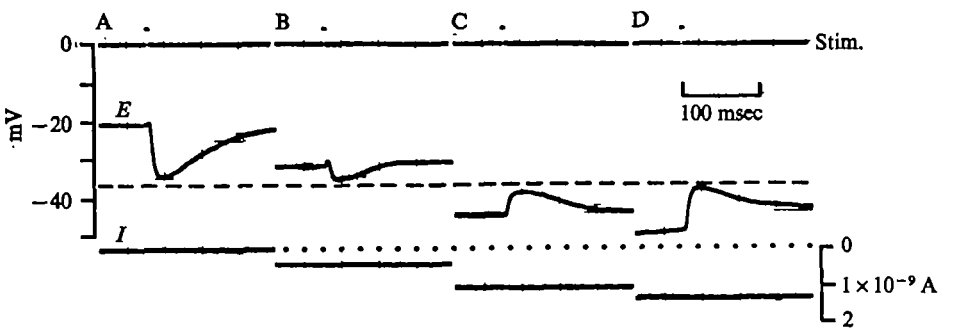

Fig. 8. Reversal level for caudal receptor potential. Specimen was bathed in 1 mM-$CaCl_2$ and 4 mM-KCl_2. Upper trace, voltage pulse applied to piezoelectric transducer. Middle trace, potential records. Lowest trace, hyperpolarizing current applied with intracellular electrode. No current applied in A. Posterior receptor potential reverses sign at about −37 mV.

The adequate stimulus

Voltage pulses with different exponential rates of rise were applied to the piezoelectric crystal which drove the stimulating probe. The rate of movement of the stylus was thus changed while the final displacement was held constant. As the stylus movement was slowed, the receptor potential declined in amplitude (Fig. 5). The rate of displacement of the probe appears to be the effective stimulus rather than the final distance through which it moves.

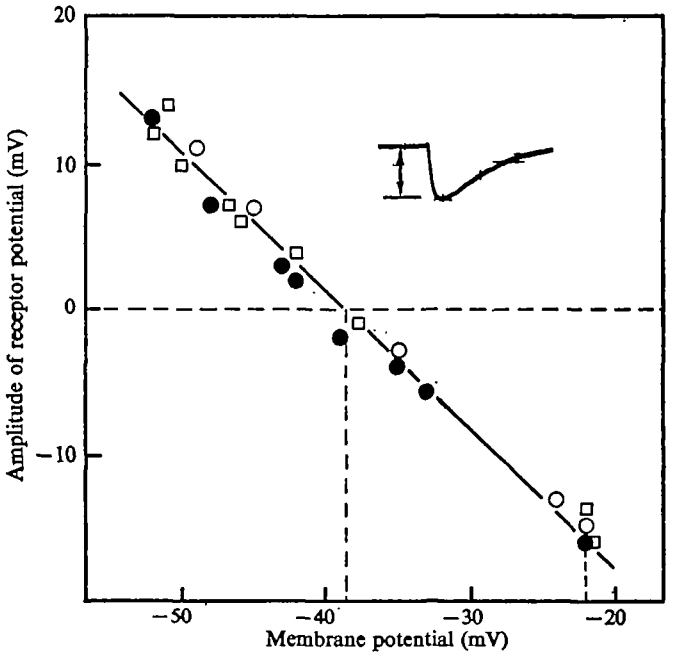

Fig. 9. Amplitude (see inset) of receptor potential plotted against membrane potential. Membrane potential shifted by means of intracellular polarizing electrode as in Fig. 6. Each symbol represents a different specimen.

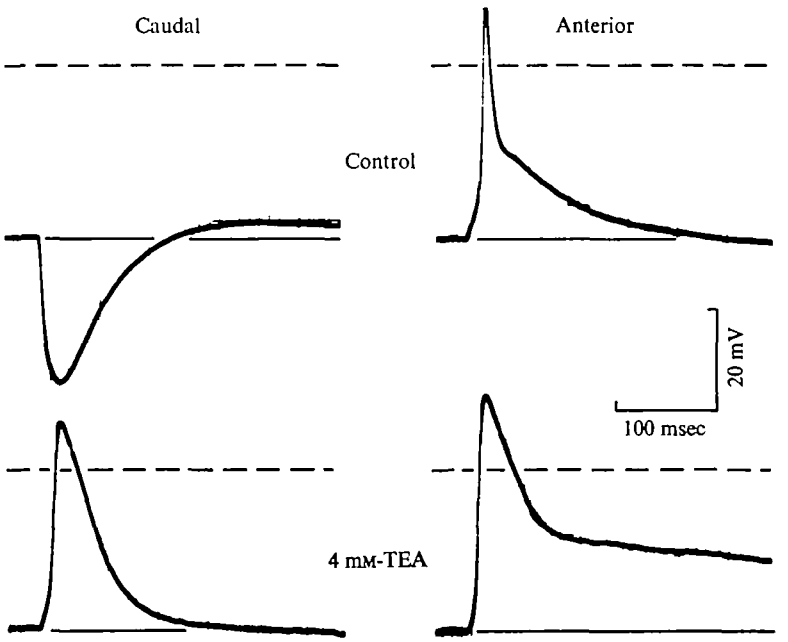

Fig. 10. Effects of tetraethylammonium bromide (TEA). Potentials evoked by stimulation of posterior (left) and anterior (right) ends of the cell in the absence (upper) and presence (lower) of 4 mM TEA. Posterior receptor potential shows reversed polarity in presence of TEA, and resembles the anterior receptor potential.

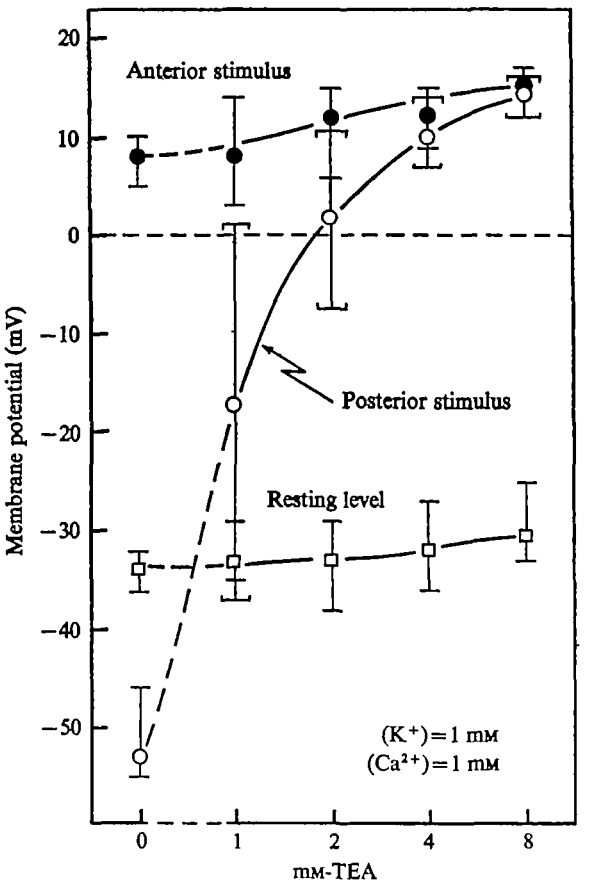

Fig. 11. Peak values of anterior and caudal responses plotted against TEA concentration. KCl and $CaCl_2$ were 1 mM each throughout. Each point on the graph is the mean of 3–6 measurements, with spread indicated.

Ionic specificity of the receptor current

The relations between membrane potentials and potassium concentrations were determined by altering extracellular potassium concentrations $(K)_0$ (Fig. 6) and evoking the maximal receptor potential with stimuli to the caudal surface. The peak of the receptor potential shifted with a positive slope of +50 mV per 10-fold increase in potassium concentration, compared to the predicted +58 mV for a highly selective potassium electrode. When $(K)_0$ was sufficiently high the polarity of the posterior response reversed (Fig. 6*f*).

Increase in $CaCl_2$ concentration produced only a slight positive shift of the maximum value attained by the receptor potential (Fig. 7). Thus neither Ca^{2+} nor Cl^- appear to provide a significant source of EMF for the receptor current.

The reversal potential was determined as shown in Fig. 8. The caudal surface was given supramaximal mechanical stimuli while current from an intracellular microelectrode hyperpolarized the membrane. The peak of the receptor potential closely approached the reversal potential over a wide range, the sign of the receptor potential changing when the membrane was polarized beyond the reversal level. The relation-

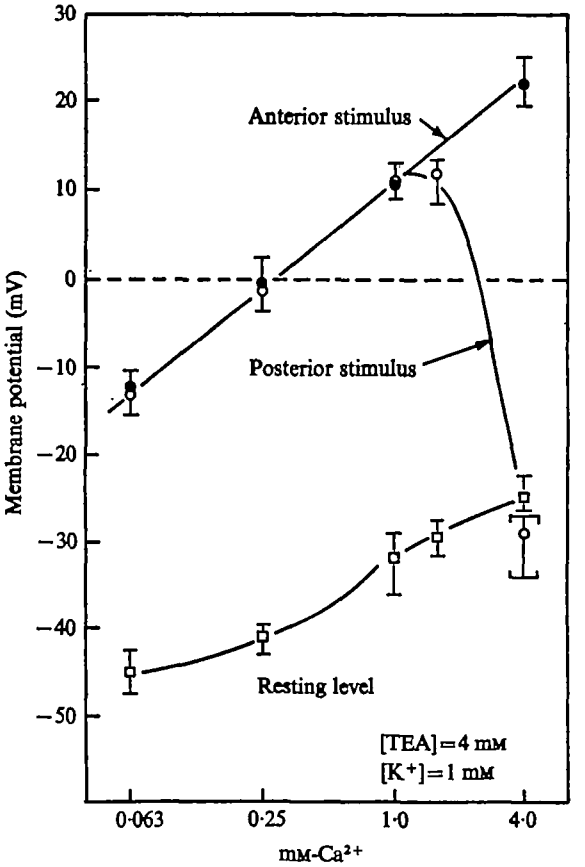

Fig. 12. Antagonism between TEA and Ca^{2+}. In the presence of 4 mM TEA the peak value of the response to stimulation of the posterior end (open circles) equals that of the response to stimulation of the anterior end (closed circles) at 1 mM-Ca^{2+} and below. Above 2 mM-Ca^{2+} the posterior response assumes its normal polarity. Each point on the graph is the mean of 3–6 measurements, with spread indicated.

ship between membrane potential and amplitude (base-to-peak) of the receptor potential was linear (Fig. 9). In a solution of 4 mM-K and 1 mM-Ca the reversal potential was −37 mV, while the resting potential was −20 mV.

In the presence of 4 mM tetraethylammonium bromide (TEA), the receptor potential produced by stimulation of the caudal region changed polarity, so that the stimulus elicited a depolarization similar to that evoked by stimulation of the anterior region of the cell (Fig. 10). As the concentration of TEA was increased from zero to 8 mM the receptor potential progressively became more positive and reversed polarity (Fig. 11). The depolarizing response then approached the level of the regenerative depolarization evoked by stimulation of the anterior surface (Eckert *et al.* 1972). When the TEA concentration was held constant at 4 mM the peak value of the depolarizing response increased with a slope of +20 mV per 10-fold rise of calcium concentration up to 1·0 mM, and lay on the same points as the response to stimulation of the anterior end (Fig. 12). At calcium concentrations beyond 2 mM the caudal

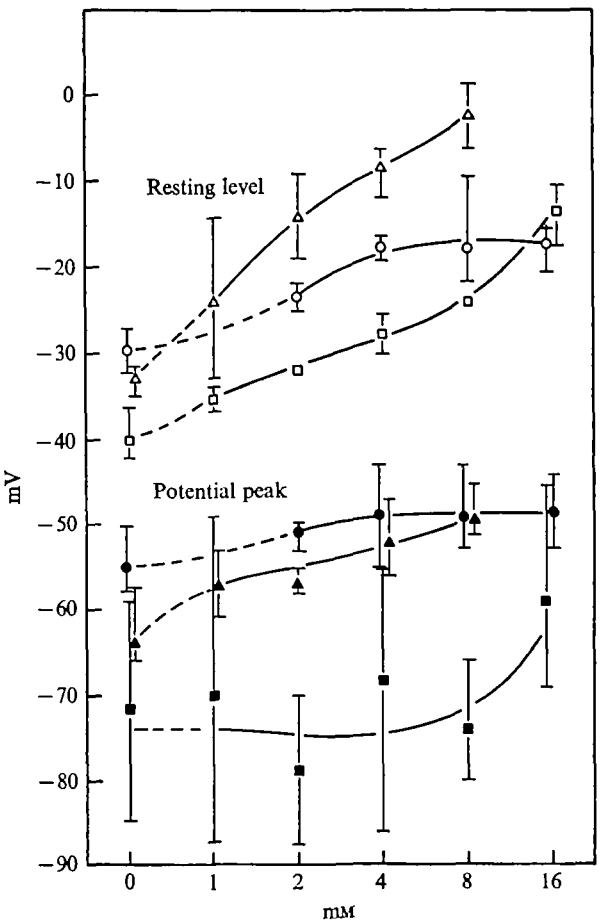

Fig. 13. Potential as a function of extracellular Na^+, Mg^{2+}, and Mn^{2+}. K^+ and Ca^{2+} held constant throughout at 1 mM each. The mean levels of resting and peak potentials in the control solution (1 mM-K + 1 mM-Ca) were different in each ionic series, because each series was carried out on a different sub-culture on a different date. Each point on the graph gives the mean and spread of 3–6 measurements made on specimens from the same subculture. ▲, Mn^{2+}; ●, Mg^{2+}; ■, Na^+. $[Ca^{2+}] = 1$ mM; $[K^+] = 1$ mM.

receptor potential regained its normal polarity. This suggests some kind of an antagonism between Ca^{2+} and TEA.

The receptor potential showed relatively little or no sensitivity to Na^+, Mg^{2+} and Mn^{2+} when the chlorides of these ions were added in concentrations up to 16 mM (Fig. 13).

DISCUSSION

Mechanical deformation of the cell membrane in the caudal region of *Paramecium* produces a K^+-dependent hyperpolarizing receptor potential. Since this accompanies an increase in input conductance of the cell, we conclude that the stimulus to the caudal end produces a local increase in conductance for K^+, which allows potassium ions to flow out through the mechanically stimulated membrane, according to their electrochemical gradient. This potassium efflux constitutes an hyperpolarizing current.

As seen in Fig. 8, the hyperpolarization closely approaches its reversal potential, indicating a strong dominance of the evoked conductances over the resting conductances. More direct measurement of the membrane conductance (Fig. 4) confirmed this. Extracellular Na^+, Ca^{2+}, Mn^{2+}, or Mg^{2+} (Fig. 7 and Fig. 13) fail to diminish the response significantly. Thus, the conductance increase evoked by mechanical stimulation of the membrane over the caudal end of *Paramecium* appears to be selective for K^+. Other mechanoreceptor potentials in which ionic mechanisms have been investigated all result from relatively nonselective increases in cation permeability (Diamond, Gray & Inman, 1958; Ottoson, 1964; Edwards, Terzuolo & Washizu, 1963).

Since the receptor current appears to be carried primarily by K^+, the reversal potential gives a minimum estimate of the equilibrium potential for potassium. Reversal of sign in the experiment of Fig. 8 occurred at about -37 mV. If it is assumed that this coincides with the equilibrium potential for K^+, the intracellular potassium activity can be calculated from the Nernst relation and the extracellular (4 mM) concentration. This gives an intracellular K^+ activity of 17·5 mM/l. In this context it is interesting that the receptor current is zero when the extracellular K^+ is adjusted to about 18 mM (Fig. 6). This is further evidence that the intracellular concentration of free potassium lies in this range.

In spite of a high degree of selectivity of the stimulated caudal receptor membrane for K^+ over the other physiological cations, some evidence suggests that mechanical stimulation of the caudal region also produces increases in permeability for ions other than potassium. Thus, the receptor potential changes sign and becomes depolarizing when TEA is added (Figs. 10 and 11). Two possible explanations for this change in direction of receptor current are: (i) TEA blocks the K^+ current and unmasks an inward current similar to the anterior receptor current (Eckert *et al.* 1972); or (ii) TEA itself carries current inward in response to the stimulus. TEA is known to carry charge across the excited membrane of lobster muscle (Werman & Grundfest, 1961). In *Paramecium* TEA applied externally reduces the repolarizing current in the regenerative calcium response (Friedman & Eckert, 1972) as it does in muscle (Stanfield, 1970). Internally or externally applied TEA also produces a 50% increase in the input resistance of *Paramecium* (Friedman & Eckert, 1972). The present evidence does not permit us to decide whether TEA carries an inward current during mechanical stimulation of the caudal membrane or whether by blocking the potassium current it unmasks an inward current carried by another ion such as Ca^{2+}. When the calcium concentration approaches and exceeds that of TEA (Fig. 10), the inward current is suppressed and the normal hyperpolarizing polarity is restored. This antagonism between TEA and Ca^{2+} remains unexplained. Depolarization evoked by a stimulus to the posterior surface in the presence of TEA has an inflexion on the upstroke similar to that seen with stimulation of the anterior surface (Fig. 10). This, plus the similarity in overshoot (Figs. 11 and 12), indicates that the depolarizing receptor current in the presence of TEA elicits the regenerative calcium response (Naitoh, Eckert & Friedman, 1972; Eckert *et al.* 1972) which accounts for most of the depolarization.

Vertebrate and molluscan photoreceptor cells have been shown to become hyperpolarized upon photic stimulation (Bortoff, 1964; Toyoda, Nosaki & Tomita, 1969;

Mpitsos, 1969; McReynolds & Gorman, 1970*a*). In the vertebrate photoreceptor the hyperpolarization results from a drop in sodium conductance, producing a shift toward the potassium equilibrium potential (Toyada *et al.* 1969). In the scallop eye the hyperpolarization results from an increased conductance (McReynolds & Gorman, 1970*b*), perhaps to K^+. A specific increase in K^+ conductance produced by mechanical stimulation has not yet been demonstrated in mechanoreceptors other than the caudal membrane of *Paramecium*.

The hair cells of vertebrate 8th nerve mechanoreceptor systems (Lowenstein & Wersall, 1959; Flock, 1965) also show hyperpolarizing and depolarizing potential shifts in response to mechanical stimulation. These, however, are specific for the direction of membrane displacement. Recent evidence (Hillman & Lewis, 1971) suggests that the membrane potential of hair cells is modulated by raising or lowering of tension applied to a single patch of cell membrane. A similar situation obtains in the nerve ending of the Pacinian corpuscle in which depolarizing and hyperpolarizing shifts of membrane potential result respectively from increasing and decreasing the resting tension of the receptor membrane. This results in the modulation of a single (set of) conductance(s) in the receptor membrane (Nishi & Sato, 1968). In contrast to these examples, the cell membrane of *Paramecium* exhibits two functionally and anatomically separate mechanotransducer regions, each responding to identical stimuli with a conductance increase selective for a different species of cation.

SUMMARY

1. Small, brief mechanical stimuli were delivered with a microstylus to the surface of *Paramecium caudatum* bathed in solutions of 1 mM-$CaCl_2$, 1 mM KCl + 1 mM Tris HCl, pH 7·2.

2. Stimulation of the caudal end produced a graded hyperpolarizing receptor potential which reached a maximum within 50 msec and decayed more slowly.

3. The input conductance at the peak of the caudal receptor potential increased to a value of at least 6 times that of the resting membrane.

4. The potential diminished in amplitude when the membrane was hyperpolarized by injected d.c. current, and reversed sign with sufficient hyperpolarization. The reversal potential in a solution of 1 mM-$CaCl_2$ + 4 mM-KCl was −37 mV, while the resting potential was −20 mV.

5. The peak of the receptor potential was shifted about +50 mV per 10-fold increase in extracellular K^+. Cl^- and Ca^{2+} and other cations produced little or no shift in the potential peak of the response. It is concluded that mechanical stimulation of the caudal surface produces a local increase in conductance, predominantly to K^+.

6. Extracellular tetraethylammonium converts the normally hyperpolarizing receptor potential to a depolarization similar to the potential produced in response to mechanical stimulation of the anterior surface. The TEA effect is antagonized by calcium ions.

This work was supported by NSF grant GB 30499 and USPHS grant NS-08364.

REFERENCES

BORTOFF, A. (1964). Localization of slow potential responses in the *Necturus* retina. *Vision Res.* **4**, 627–35.

DIAMOND, J., GRAY, J. A. B. & INMAN, D. R. (1958). The relation between receptor potential and the concentration of sodium ions. *J. Physiol.* **142**, 382–94.

ECKERT, R. (1965). Bioelectric control of bioluminescence in the dinoflagellate *Noctiluca*. *Science* **147**, 1140–5.

ECKERT, R. (1972). Bioelectric control of ciliary activity. *Science* **176**, 473–81.

ECKERT, R. & NAITOH, Y. (1970). Passive electrical properties of *Paramecium* and problems of ciliary coordination. *J. gen. Physiol.* **55**, 467–83.

ECKERT, R. & NAITOH, Y. (1972). Bioelectric control of locomotion in the ciliates. *J. Protozool.* **19**, 237–43.

ECKERT, R., NAITOH, Y. & FRIEDMAN, K. (1972). Sensory mechanisms in *Paramecium*. I. Two components of the electric response to mechanical stimulation of the anterior surface. *J. exp. Biol.* **56**, 683–94.

EDWARDS, C., TERZUOLO, C. A. & WASHIZU, Y. (1963). The effect of changes in the ionic environment upon an isolated crustacean sensory neuron. *J. Neurophysiol.* **26**, 948–57.

FLOCK, A. (1965). Transducing mechanisms in the lateral line canal organ receptors. *Cold Spring Harbor Symp. Quant. Biol.* **30**, 133–45.

FRIEDMAN, K. & ECKERT, R. (1972). Ionic and pharmacological modification of input resistance and excitability in *Paramecium*. *Comp. Biochem. Physiol.* (In the Press.)

HILLMAN, D. E. & LEWIS, E. R. (1971). Morphological basis for a mechanical linkage in otolithic receptor transduction in the frog. *Science* **174**, 416–19.

JENNINGS, H. S. (1906). *Behavior of the Lower Organisms*. New York: Columbia University Press.

KINOSITA, H., DRYL, S. & NAITOH, Y. (1964). Relation between the magnitude of membrane potential and ciliary activity in *Paramecium*. *J. Fac. Sci. Univ. Tokyo Sect. IV*, **10**, 303–9.

LOWENSTEIN, W. & WERSALL, J. (1959). A functional interpretation of the electron-microscopic structure of the sensory hairs in the cristae of the elasmobranch *Raja clavata* in terms of directional sensitivity. *Nature, Lond.* **184**, 1807–10.

MCREYNOLDS, J. S. & GORMAN, A. L. F. (1970*a*). Photoreceptor potentials of opposite polarity in the eye of the scallop, *Pecten irradians*. *J. gen. Physiol.* **56**, 376–91.

MCREYNOLDS, J. S. & GORMAN, A. L. F. (1970*b*). Membrane conductances and spectral sensitivities of *Pecten* photoreceptors. *J. gen. Physiol.* **56**, 392–406.

MPITSOS, G. J. (1969). An electrophysiological analysis of vision in a file clam (*Lima scabra* Born). Ph.D. dissertation, University of Virginia.

NAITOH, Y. (1958). Direct current stimulation of *Opalina* with intracellular microelectrode. *Annot. Zool. Japan* **31**, 59–73.

NAITOH, Y. & ECKERT, R. (1968). Electrical properties of *Paramecium caudatum*: Modification by bound and free cations. *Z. vergl. Physiol.* **61**, 427–62.

NAITOH, Y. & ECKERT, R. (1972). Electrophysiology of ciliate protozoa. In *Exp. in Physiol. and Biochem.*, vol. 5 (G. A. Kerkut, ed.). London: Academic Press.

NAITOH, Y. & ECKERT, R. (1969*a*). Ionic mechanisms controlling behavioral responses of *Paramecium* to mechanical stimulation. *Science* **164**, 963–5.

NAITOH, Y. & ECKERT, R. (1969*b*). Ciliary orientation: Controlled by cell membrane or by intracellular fibrils? *Science* **166**, 1633–5.

NAITOH, Y., ECKERT, R. & FRIEDMAN, K. (1972). A regenerative calcium response in *Paramecium*. *J. exp. Biol.* **56**, 667–82.

NEW, W., Jr. (1972). Constant current source for microelectrodes. *J. appl. Physiol.* **32**, 885–7.

NISHI, N. & SATO, M. (1968). Depolarizing and hyperpolarizing receptor potentials in the non-myelinated nerve terminal in Pacinian corpuscles. *J. Physiol.* **199**, 383–96.

OTTOSON, D. (1964). The effect of sodium deficiency on the response of the isolated muscle spindle. *J. Physiol.* **171**, 109–18.

STANFIELD, P. R. (1970). The effect of the tetraethylammonium ion on the delayed currents of frog skeletal muscle. *J. Physiol.* **209**, 209–29.

TOYODA, J. I., NOSAKI, H. & TOMITA, T. (1969). Light-induced resistance changes in single photoreceptors of *Necturus* and *Gekko*. *Vision Res.* **9**, 453–63.

WERMAN, R. & GRUNDFEST, H. (1961). Graded and all-or-none electrogenesis in arthropod muscle II. The effects of alkali-earth and onium ions on lobster muscle fibers. *J. gen. Physiol.* **44**, 997–1027.

WOOD, D. (1970). Electrophysiological studies of the protozoan *Stentor coeruleus*. *J. Neurobiol.* **1**, 363–77.