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A REGENERATIVE CALCIUM RESPONSE IN *PARAMECIUM*

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INTRODUCTION

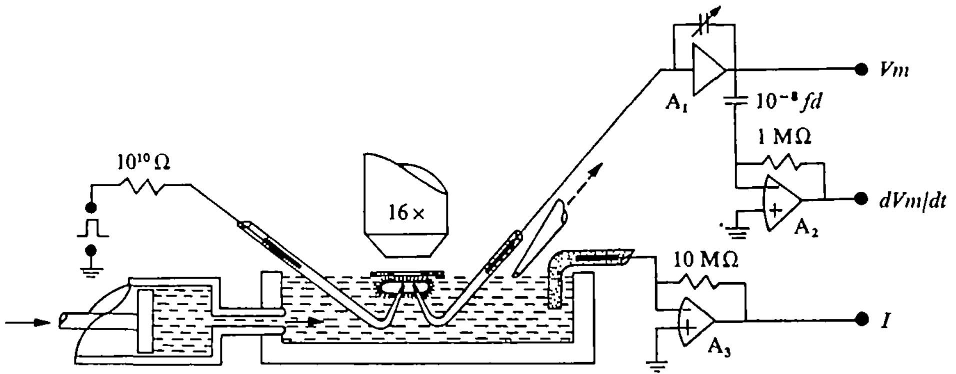
The electric properties of *Paramecium* have gained interest because of the emerging role of membrane potential and conductance in the control of ciliary activity (Kinosita, 1954; Okajima, 1953; Naitoh, 1958; Kinosita, Murakami & Yasuda, 1965; Naitoh & Eckert, 1969*a*, *b*; Eckert & Naitoh, 1970). Recent evidence indicates that ciliary responses are coupled to membrane potential by membrane-regulated calcium fluxes (Eckert, 1972). Membrane potential changes occur in response to mechanical stimuli (Naitoh & Eckert, 1969*a*, *b*), radiant energy (Hildebrand, 1970), and changes in extracellular electrolyte concentration (Kamada, 1934; Naitoh & Eckert, 1968*a*). Further interest comes from the isolation of behavioural mutant of *Paramecium* (Kung, 1971) which has lost the property of regenerative depolarization characteristic of the wild-type membrane (Kung & Eckert, 1972).

The cell membrane of *Paramecium* normally produces a graded, spike-like, regenerative response to applied outward current (Eckert & Naitoh, 1969). This is followed closely in time by a re-orientation of the cilia so that the direction of the effective stroke (and, hence, the direction of swimming) is reversed (Eckert & Naitoh, 1970). A similar graded response is produced by the depolarization in response to mechanical stimulation of the anterior surface of *Paramecium* (Eckert, Naitoh & Friedman, 1972). In this paper we present evidence that the graded response is dependent on extracellular Ca^{2+}, and propose that the membrane of *Paramecium* undergoes an increase in calcium permeability in response to depolarization, which permits Ca^{2+} to flow inward down its electrochemical gradient. The regenerative response in *Paramecium* is similar in this and several other respects to the graded, non-propagated responses produced by crustacean muscle (Fatt & Katz, 1953; Fatt & Ginsborg, 1958; Werman & Grundfest, 1961).

METHODS

Specimens of *P. caudatum* were obtained from General Biological Supply Inc., and were cultured in a hay infusion as described elsewhere (Naitoh & Eckert, 1972).

Instrumentation and techniques employed for stimulation and recording were similar in most respects to those described earlier (Naitoh & Eckert, 1968*a*; Eckert & Naitoh, 1970; Naitoh & Eckert, 1972). Cells were stimulated with depolarizing pulses delivered through intracellular electrodes with tip diameters below 1 μm. The stimulating and recording system is shown diagrammatically at the top of Fig. 1. Glass capillary microelectrodes filled with 1/10 M-KCl and having resistances of 100–300 megohms were used for recording, and similar electrodes filled with 1/10 or 3 M-KCl



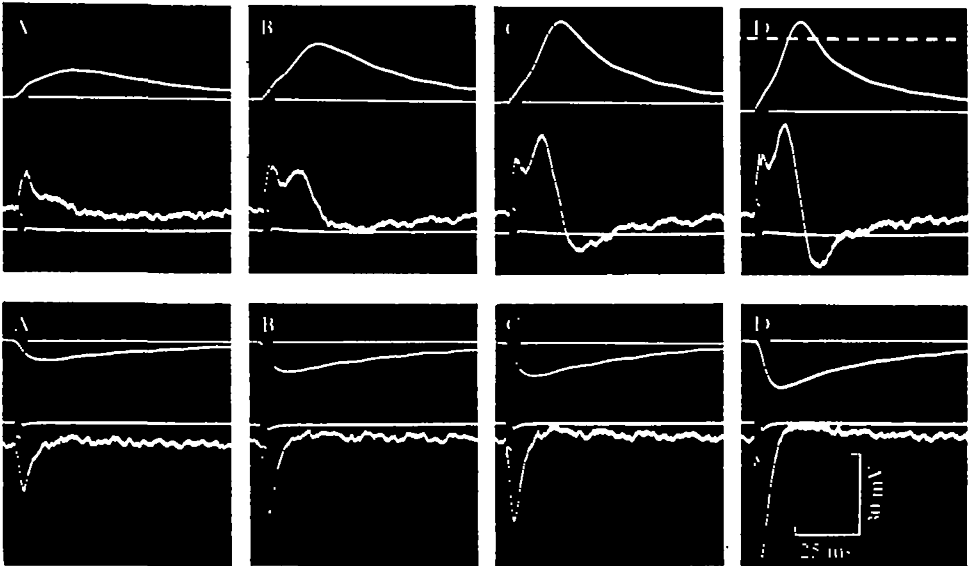


Fig. 1. Top, Electrical instrumentation. Current pulses were passed into *Paramecium* through one electrode, and membrane potential, V_m, was recorded with a second electrode. Head stage A_1 had 10^{12} ohms input resistance and capacity neutralization. A_2, operational amplifier used to differentiate output of A_1. A_3, operational amplifier used to monitor current injected by current electrode. A 3 M-KCl agar bridge connected the bath to the summing junction of A_3 through a triggered calibrator.

Bottom, A–D, Membrane responses to 2 msec depolarizing current pulses. Upper trace, membrane potential; second trace, first time derivative (dV_m/dt) of V_m; lowest trace, stimulating current. Note inflection on upstroke during depolarization. A′–D′, same as A, but hyperpolarizing pulses. Sensitivity of dV_m/dt trace is 2·5 V/sec for the height of the vertical calibration line in D′. Dashed line in Fig. D indicates zero potential.

were used for passing current (Naitoh & Eckert, 1972). A Bioelectric Instruments NF1 neutralized capacitance amplifier was used for recording membrane potentials. Capacitance neutralization was adjusted by 'squaring up' a triggered calibration pulse inserted between virtual ground of the current-measuring amplifier and the bath. Potential signals were differentiated electronically with an operational amplifier and a passive network of 10 msec time constant. Reference potentials were noted after removal of the recording electrode from the organism. Except when noted otherwise,

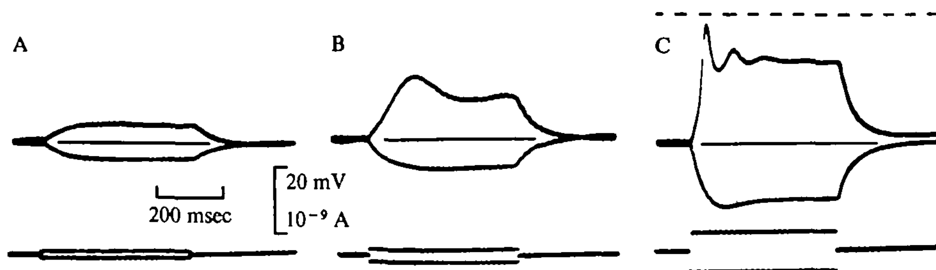


Fig. 2. Membrane responses to long current pulses. A–C, increasing current intensities. Record A shows a nearly pure electrotonic depolarization with almost no regenerative component. Regenerative behaviour increases in a graded manner as passive depolarization increases in rate of rise and amplitude in B and C. Hyperpolarization shows delayed anomalous rectification. Dashed line in C gives level of reference (zero) potential.

stimuli consisted of 1–2 msec depolarizing pulses delivered intracellularly with the current electrode. Current intensities sufficient to elicit a maximal regenerative response (at right end of curves in Fig. 3) were used unless noted otherwise.

Cells were bathed in solutions of KCl plus $CaCl_2$ except where chlorides of other cations were added in place of KCl as noted. Experimental solutions were all buffered at pH 7·2 in 1 mM Tris-HCl. Before use in experiments, specimens were washed and equilibrated for 30 min in a solution of 4 mM-KCl, 1 mM-$CaCl_2$ and 1 mM Tris-HCl.

The high tip resistance plus a large capacitance to ground along the length of electrode shaft immersed in the bath (Fig. 1), caused the current out of the *tip* of the stimulating electrode to rise and decay with a time constant of several milliseconds even though the total current (resistive current through electrode tip plus capacitive current through glass wall) monitored out of the bath rose and fell abruptly. The current trace for this reason does not give an accurate indication of the time-course of the current injected into the cell. This can be seen in Fig. 1 A′–D′, where electrotonic hyperpolarizing potentials reach their peaks with considerable delay after the end of the recorded current pulses.

RESULTS

The regenerative response

The response of the membrane to maintained pulses of current lasting about 400 msec is shown in Fig. 2. With small currents (producing several millivolts potential displacement) both depolarizing and hyperpolarizing potential shifts occur as simple exponentials with time constants in the range of 40–50 msec. In this region the current–voltage curve is essentially linear (Naitoh & Eckert, 1968*a*), suggesting that the membrane at zero current acts as a simple leaky condenser of fixed resistance and capacitance. Stronger hyperpolarization produces some delayed anomalous rectification seen as a decrease in the IR drop produced by the current passed across the membrane. With stronger depolarizing current an inflexion appears on the rising phase where a regenerative response develops out of the electrotonic potential change. After reaching a peak, the potential drops to a lower level with a damped oscillation. The maximum rate of rise and amplitude of the regenerative component increase, and its

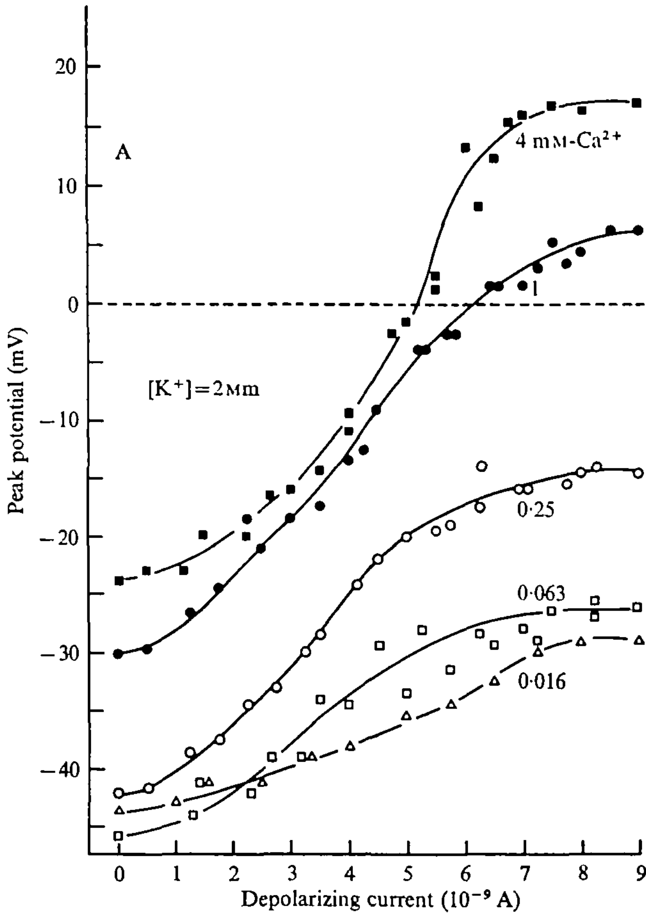


Fig. 3 A. For legend see facing page.

duration decreases, with an increase in intensity of the depolarizing current (Fig. 2B and C).

In the remaining experiments brief (1–2 msec) pulses of depolarizing current were used.

Electrical responses of the cell to brief current pulses in 1 mM-KCl + 1 mM-$CaCl_2$ are shown in Fig. 1. In the hyperpolarizing direction (A′ to D′) there is a simple electrotonic potential change. The response to a depolarizing current pulse (Fig. 1 A–D) consists of an electrotonic component which gives rise to a regenerative response, as indicated by an inflexion on the upstroke. Displays of time derivatives (dV_m/dt) show the two components of the upstroke compared with the single component during hyperpolarization. The initial component represents the rise of the electrotonic potential. At the lowest current intensity (Fig. 1 A) the electrotonic potential gives rise to a regenerative component which is barely detectable. As the current intensity is increased, this component shows a graded increase to a maximum overshoot of 15 mV and a maximum rate of rise of 7 V/sec (Fig. 1 B–D).

The peak potential of the response has a sigmoid relation to the intensity of the brief (2 msec) current pulse used to stimulate the cell. The peak potential shows little

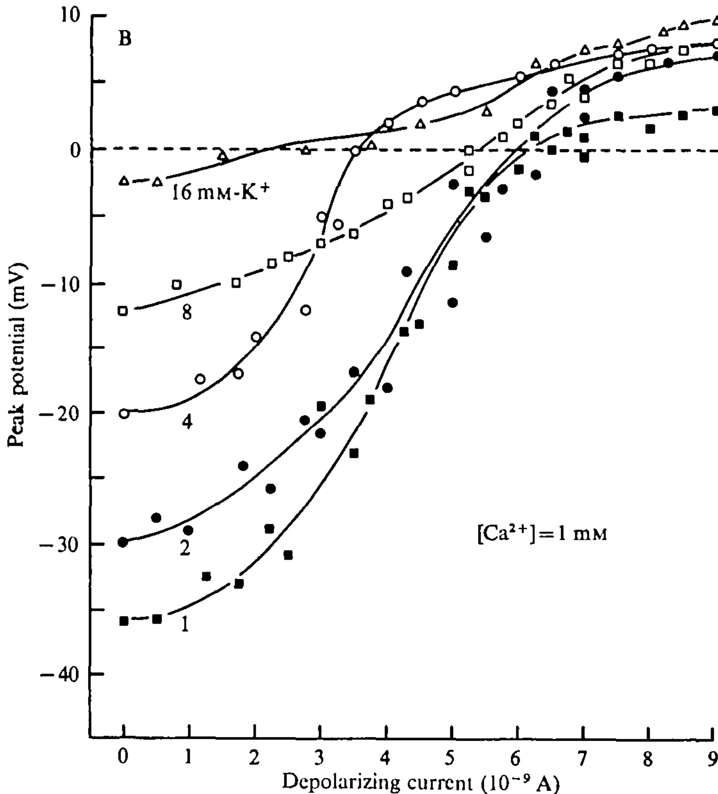


Fig. 3. Peak value of membrane potential attained by regenerative depolarization plotted against intensity of 2-msec pulses of depolarizing current. Peak of response came after current pulse ended. A, potassium held constant at 2 mM throughout while extracellular calcium was altered between 0·016 and 4 mM. Peak value became saturated with stimulus currents of somewhat less than 10^{-8} amps at levels dependent on concentration of calcium. B, same as A, but calcium held constant at 1 mM while potassium concentration was altered.

further increase at 2 msec currents above 7×10^{-9} A (Fig. 3). In experiments that follow we stimulated with current pulses of supramaximal intensity (right-hand end of abscissae in Fig. 3) in order to minimize variations of response amplitude produced by differences in stimulus currents. Along with the increased amplitude of response with increased current intensity there is a corresponding shortening of the response (Figs. 1 C and D) seen as a decrease in the time from maximum rate of rise to maximum rate of fall.

The response to a current pulse can be suppressed by hyperpolarizing the membrane with current from the intracellular polarizing electrode (Fig. 4). A hyperpolarization of several millivolts produced a noticeable drop in overshoot and a slowing of the response (Fig. 4B). The regenerative component was completely depressed with a sufficient hyperpolarization, revealing the simple electrotonic response to the brief current pulse (Fig. 4D). The depression of excitation could now be overcome by simply increasing the current intensity of the brief stimulus pulse. However, this always produced a graded response and never an all-or-none action potential, ruling out the possibility that the membrane normally fails to respond in an all-or-none manner due to partial inactivation at the resting potential.

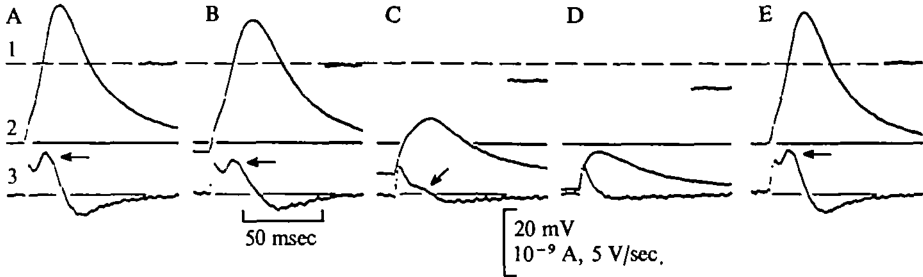


Fig. 4. Suppression of response by hyperpolarization. Recording A shows potential response (trace 2) to 2 msec current pulse applied at resting membrane potential. Dashed line 1 gives reference level for membrane potential, and also the polarizing current. Recordings B–D obtained in response to similar 2 msec depolarizing stimulus current pulse added to steady hyperpolarizing current used to produce a negative shift in membrane steady potential. Short segment of current trace at right side of each recording indicates level of applied current with respect to dashed line zero reference. Recording E shows recovery of normal response when polarizing current is removed. Note reduction and disappearance of regenerative component with hyperpolarization. Arrows in derivative recording (trace 3) point to deflexions corresponding to regenerative upstroke in trace 2.

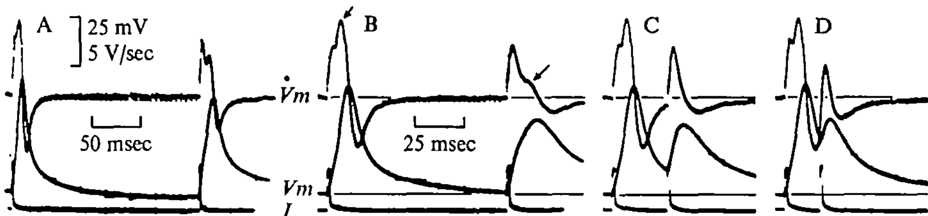


Fig. 5. Refractoriness of regenerative response. A pair of 2 msec stimuli of varying interpulse interval were delivered every 20 sec. Arrows in derivative recordings indicate deflexions corresponding to regenerative upstroke. Recording A made with slower sweep than B–D.

When two stimuli were delivered with an interval of less than 250 msec the amplitude and rate of rise of the second response was reduced (Fig. 5A and B). The maximum rate of rise of the regenerative component (arrow) was reduced by more than 75% when the interval was 80 msec (Fig. 5B). With intervals of less than 30 msec (C–D) the regenerative component was absent, as judged from the lack of an inflexion on the rising phase of the second depolarization.

Inactivation of the response was examined by stimulating with a 2 msec current pulse toward the end of a long (300 msec) conditioning pulse (Fig. 6). In recording A, with no conditioning current, the membrane was at resting potential. The current pulse then elicited a maximal response. When the stimulus pulse was preceded by a conditioning pulse which depolarized the membrane by about 23 mV (Fig. 6B) only a trace of the regenerative compartment remained. This component failed completely (but reversibly) with further conditioning depolarization (Fig. 6C), leaving only a small electrotonic response. It is evident that for a stimulus pulse of given current intensity, both electrotonic and regenerative components of the response diminish in amplitude during steady depolarization. This is consistent with the drop in resistance which occurs with depolarization (Naitoh & Eckert, 1968*a*), and with an inactivation of the membrane potential-dependent calcium conductance analogous to the inactivation of potential-dependent sodium conductance in nerve membrane produced by a conditioning depolarization (Hodgkin, 1957).

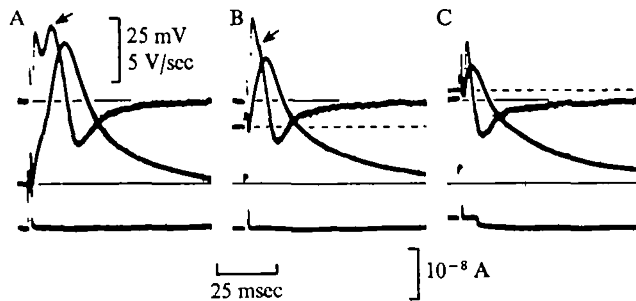


Fig. 6. Effect of depolarization on regenerative behaviour. A, normal response to 2 msec stimulus pulse, no conditioning current used. B, cell depolarized about 23 mV by 300 msec conditioning current. Stimulus pulse as in A added just before termination of conditioning depolarization. Regenerative component is greatly depressed by steady depolarization. C, regenerative component completely abolished by further membrane depolarization.

Ionic mechanisms

Increasing the concentrations of KCl or $CaCl_2$ depolarizes the membrane with a slope which is about half that predicted from the Nernst equation for either a pure K^+ or a pure Ca^{2+} electrode (Figs. 7, 8). Substitution of Cl^- with a non-permeable anion such as propionate produced no significant change in resting potential (Fig. 9), indicating that chloride either penetrates very slowly or produces a transient potential shift which escapes the time resolution of our recording during solution changes.

While the resting membrane shows a rather non-specific permeability for various cations (Naitoh & Eckert, 1968*a*), the membrane appears to undergo a differential transient increase in permeability to Ca^{2+} when suddenly depolarized. This is inferred from the data in Figs. 7 and 8. The membrane was stimulated with a 2 msec pulse of current sufficient to evoke a maximum regenerative response (toward right end of current scale in Fig. 3). The peak of the response reaches potentials of increasing positivity as $[Ca]_0$ is raised (Figs. 3A and 8). The peak shows an increment of 25 mV per 10-fold increase in $[Ca]_0$ compared to 29 mV predicted by the Nernst relation.

The peak potential shows a small positive increment (about 9 mV/10-fold increase) with increased $[K]_0$ (Fig. 7). This limited effect rules out K^+ as the major source of regenerative inward current. Moreover, K^+ cannot carry inward current since the equilibrium potential for potassium is more negative than the resting potential (Eckert *et al.*, 1972). The effect of increased KCl concentration cannot result from changes in chloride concentration, since (1) the small increment in overshoot with increasing $[KCl]_0$ is in the positive-going direction, while the concomitant shift in chloride equilibrium potential is negative, and (2) the resting potential and overshoot remained unchanged when propionate replaces Cl^- (Fig. 9). With K^+ and Cl^- both eliminated above as carriers of inward current, calcium remains the only obvious source of charge to carry the inward current. This conclusion is supported by the predominant calcium dependence of the overshoot.

Inspection of the electrical responses (Figs. 7, 8 and 10A) indicates that the base-to-peak amplitude and rate of rise are not simple functions of extracellular [Ca], but are more closely related to the relative concentrations of Ca^{2+} and K^+. Decreasing $[K]_0$ and increasing $[Ca]_0$ both result in greater amplitude of the regenerative response.

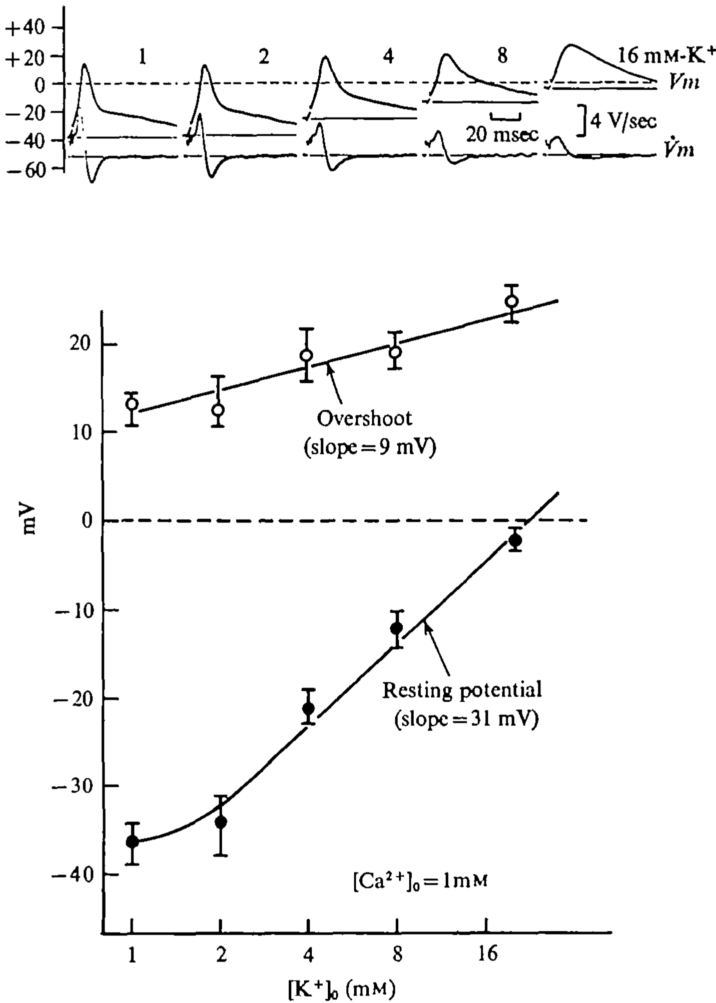


Fig. 7. Electrical response as function of extracellular potassium. Calcium held constant at 1 mM throughout; $[K]_0$ varied. Stimuli consist of 2 msec current pulses of supra-maximal intensity. Top, potential (V_m) and derivative ($\dot{V}_m$) recordings at KCl concentrations indicated. Each point on graph is the mean of five measurements with deviations.

Maintaining a simple proportionality between $[K]_0$ and $[Ca]_0$ still leads to widely diverging responses. Approximately similar amplitudes of response are obtained at similar $[K]/[Ca]^{\frac{1}{2}}$ ratios (Fig. 10). The agreement is best at higher relative Ca^{2+} concentrations. When $[Ca]_0$ drops below 10^{-4} M the amplitude drops disproportionately, since the peak potential continues to drop with decreased $[Ca]_0$ while the resting potential fails to show further increase in negativity (Fig. 8).

Repolarization following excitation takes place in two stages as seen in Figs. 7, 8, 9 and 12. Following an initial rapid repolarization there is a slower phase of repolarization. The latter more closely resembles the time constant of the electrotonic response to current, and is therefore judged to be due to recharging of the membrane capacitance through the resting conductance.

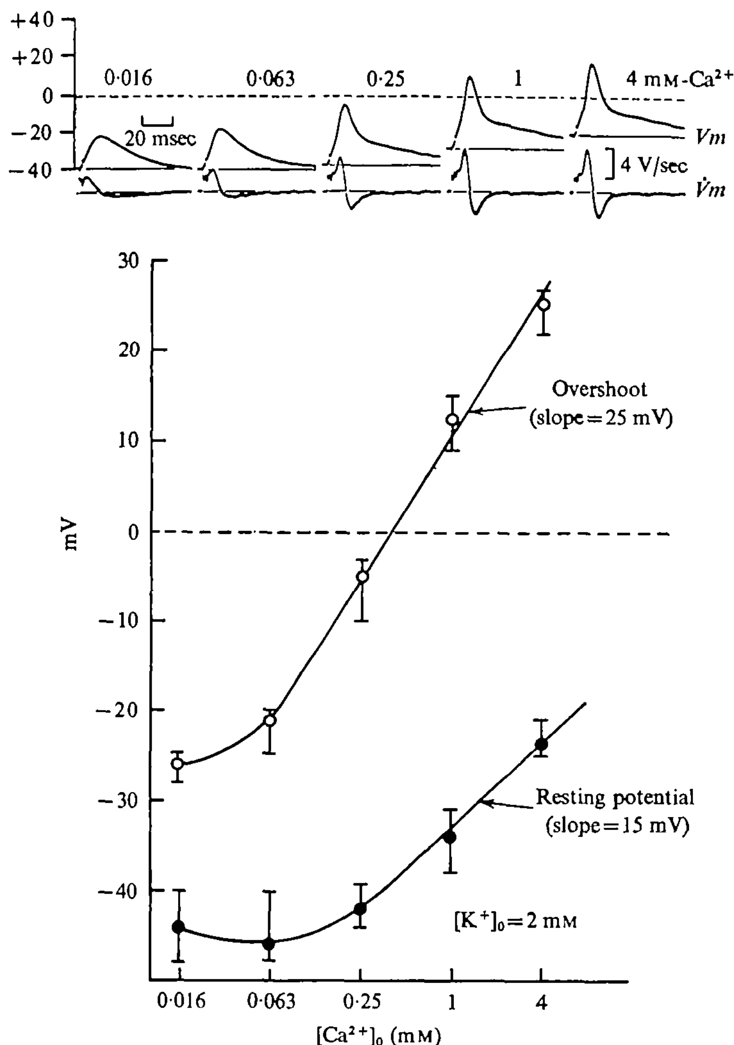


Fig. 8. Electrical response as function of extracellular calcium. Potassium was held constant at 2 mM while $CaCl_2$ concentration was varied as shown. Details same as in Fig. 7.

Selectivity of the membrane

Other cations were tested to determine their ability to substitute for, or interfere with, calcium as carrier of the regenerative current. Test solutions consisted of the chloride salt of the cation plus 1 mM-$CaCl_2$ and 1 mM Tris buffer. A total of ten cations were tested at concentrations of 1, 4, and 16 mM (Fig. 11). All of them partially depolarized the membrane, in agreement with earlier observations (Kamada, 1934; Naitoh & Eckert, 1968*a*). Li^+ and Na^+ had no effect on the overshoot of the regenerative response elicited by a 2 msec current pulse. Rb^+, Cs^+, NH_4^+, Mg^{2+}, and TEA^+ (tetraethylammonium bromide) all produced small increments in overshoot similar to that produced by increase in extracellular potassium (Fig. 7). None of these ions produced an increment in overshoot as great as, or significantly greater than, the reduction in resting potential. The only ion which produced a significant increase in overshoot was

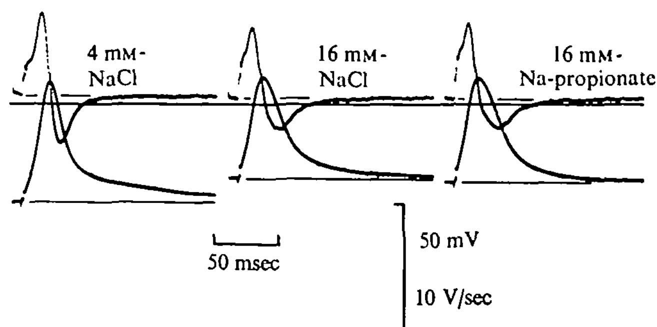


Fig. 9. Chloride substitution. Increasing NaCl caused some steady depolarization and some slowing of response. This was due entirely to the cation, since replacing Cl^- with propionate produced no change in electrical behaviour. 1 mM-$CaCl_2$ throughout.

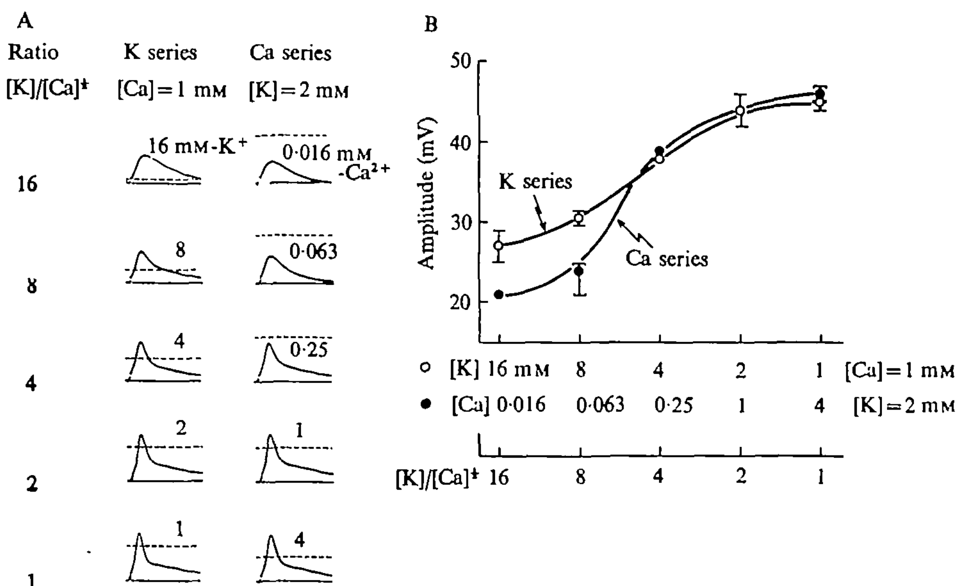


Fig. 10. Amplitude of response at various ratios of $[K]/[Ca]^{\frac{1}{2}}$. Data from Figs. 7 and 8. A, responses compared in solutions of widely divergent ionic strengths. Each horizontal pair recorded in test media of identical ratio $[K]/[Ca]^{\frac{1}{2}}$ as indicated on extreme left. Dashed lines indicate zero membrane potential. B, Base-to-peak amplitudes plotted against $[K]_0$, $[Ca]_0$ and $[K]/[Ca]^{\frac{1}{2}}$. Black circles, $[K]_0$ held constant at 2 mM, $[Ca]_0$ varied. Open circles, $[Ca]_0$ held constant at 1 mM, $[K]_0$ varied. Each point gives the average of five measurements on five specimens, with deviations.

Mn^{2+} (Figs. 11 and 12), with an increment of about 17 mV per 10-fold increase in $[Mn]_0$. Effects of Ba^{2+}, Sr^{2+} and La^{3+} on the electrical response are described elsewhere (Naitoh & Eckert, 1968*b*; Friedman & Eckert, 1972).

It is difficult to assess competitive blocking of the regenerative current by cations, since high concentrations of cations depolarize the membrane, and this depresses the response (Fig. 6). None of the ions tested interfered strongly with the regenerative component.

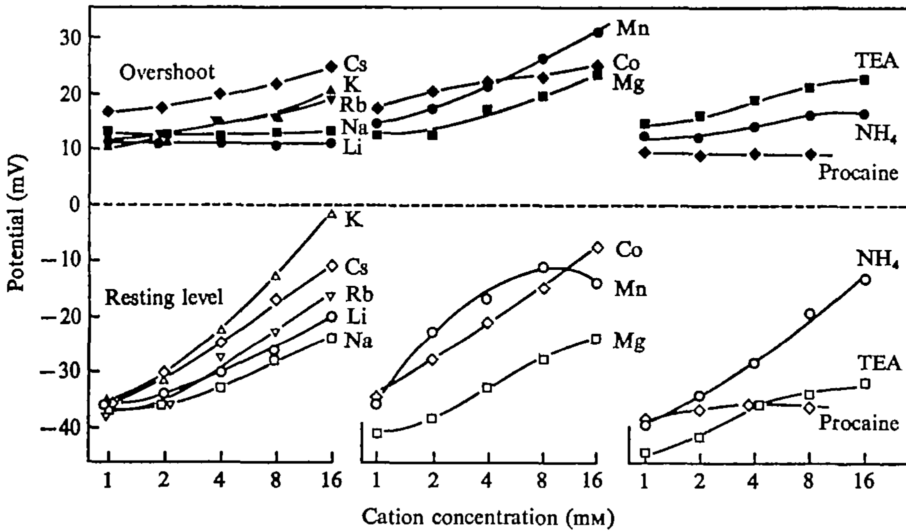


Fig. 11. Overshoot and resting potential as functions of various ion concentrations. Maximal electrical responses were evoked throughout by 2-msec depolarizing pulses. $CaCl_2$ held at 1 mM in all solutions and chloride salts of cations used throughout, with the exception of tetraethylammonium bromide (TEA). Each point gives the mean of five measurements.

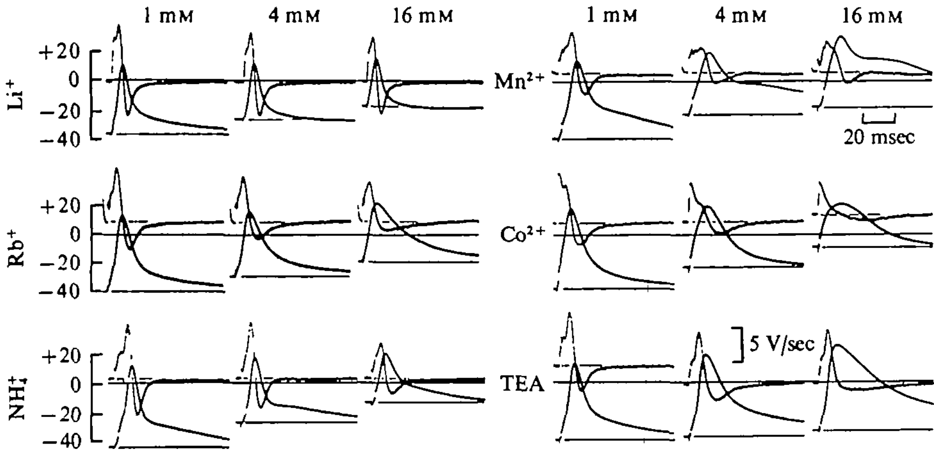


Fig. 12. Effect of various cations on electric responses. Response evoked by supramaximal 2 msec depolarizing pulses. Upper trace shows time-derivative recording of potential recorded in lower trace. Concentration of test ions given at head of each vertical column. $CaCl_2$ was held constant at 1 mM throughout.

DISCUSSION

Regenerative responses in *Paramecium* resemble those of arthropod muscle fibres (Fatt & Katz, 1953; Fatt & Ginsborg, 1958; Werman, McCann & Grundfest, 1961; Werman & Grundfest, 1961), in which the surface membrane exhibits membrane potential-dependent permeability increases to the divalent alkali-earth cations. Further similarities exist with the 'calcium response' seen in nerve terminals after the Na system is suppressed with tetrodotoxin and delayed rectification is reduced with TEA (Katz & Miledi, 1969). Although the response in *Paramecium* is graded instead of all-

or-none, and shows no clear threshold, it exhibits certain properties characteristic of conventional action potentials. These include regenerative depolarization (Fig. 1), refractoriness (Fig. 5), and repolarization which is faster than expected from the time constant of the resting membrane.

The overshoot of the graded regenerative response increases linearly with the logarithm of the extracellular calcium concentration with a slope approaching that predicted by the Nernst relation. It is insensitive to the chloride concentration, and shows only a small increment with addition of monovalent cations when $[Ca]_0$ is held constant. Ba^{2+} and Sr^{2+} are the only cations which substitute effectively for calcium in carrying the regenerative inward current in *Paramecium* (Naitoh & Eckert, 1968*b*). None of the cations tested produced a strong reduction in the response that could not be ascribed to inactivation by membrane depolarization.

Since the overshoot of the response is most closely related to the extracellular Ca^{2+} (Ba^{2+}, Sr^{2+}) concentration, we propose that depolarization of the membrane produces an increase in permeability to the alkali–earth cations. In our recording medium (and in pond water) this leads to an inward current carried by Ca^{2+} in accord with the ionic hypothesis (Hodgkin, 1957). This requires that Ca^{2+} has an electrochemical gradient which will drive it into the cell. The intracellular activity of Ca^{2+} has not been measured directly but is assumed to be several orders of magnitude below the extracellular concentrations (0·016 to 4 mM) used in these experiments. This is supported by recent experiments with extracted models of *Paramecium* (Naitoh and Kaneko, 1972) in which the ATP-activated cilia reverse direction of beating so as to produce backward swimming of the extracted model when [Ca] is raised above 10^{-6} M/l. Below this concentration the cilia produce normal forward locomotion which occurs in the living cell when the membrane is in the resting (unexcited) state. If the intracellular concentration of free Ca^{2+} is 10^{-7} M/l, the calcium equilibrium potential should range in Fig. 8 from +64 mV at the low end of the abscissa ($[Ca]_0$ = 0·016 mM) to +133 mV at the high end ($[Ca]_0$ = 4 mM). The overshoot comes no closer than about 100 mV to these values. This may be due to a dominance of potassium conductance, g_K, even when the membrane conductance to Ca^{2+} is increased during excitation.

The graded relation of the regenerative response to depolarizing stimuli is consistent with a small increase in g_{Ca} relative to g_K during excitation. It remains uncertain whether the weak graded nature of the response results primarily from a small increase in calcium conductance with depolarization, or from an early rise in potassium conductance which short circuits most of the inward calcium current during excitation. The latter was found to be the cause of graded calcium responses in crustacean muscle (Hagiwara, Hayashi & Takahashi, 1969). Suppression of the early potassium conductance in barnacle muscle with procaine produced an increase in the amplitude of the graded spike to a given stimulating current. Further evidence suggesting a short-circuiting outward potassium current is seen in the small but significant sensitivity of the peak potential to the extracellular concentration of K^+ (also Rb^+ and Cs^+) apparent in Figs. 7, 11 and 12. It appears that an increase in extracellular K^+ (Rb^+, Cs^+) decreases outward current carried through potassium-permeable channels which partially short circuits the regenerative inward calcium current. Hagiwara, Chichibu & Naka (1964) have shown that the peak potential of the Ca spike in barnacle muscle is a linear function of $[Ca]_{out}/[K]_{in}$ when $[Ca]_{in}$ is below 10^{-7} M.

In the presence of extracellular Ba^{2+} or Sr^{2+} the membrane generates all-or-none action potentials (Naitoh & Eckert, 1968*b*). This is due at least in part to an increase in resting membrane resistance (Naitoh & Eckert, 1968*a*, Figs. 17, 18 and 21), and reduced short circuiting of the regenerative inward current, as Ba^{2+} is known to do in crustacean muscle (Werman & Grundfest, 1961). Decreased potassium conductance is probably a factor in the Ba^{2+}-dependent increase in duration of the action potential (Naitoh & Eckert, 1968*b*, Figs. 5, 6). A second factor in the conversion of graded responses to all-or-none by Ba^{2+} and Sr^{2+} is their ability to substitute for Ca^{2+} in carrying the regenerative current. Thus, in the presence of 1 mM Ba^{2+} the overshoot increases by less than 5 mV per 10-fold increase in $[Ca]_0$. With $[Ca]_0$ held constant at 1 mM, the overshoot increases 22 mV per 10-fold rise in [Ba] (Naitoh and Eckert, 1968*b*, Figs. 5, 6). This indicates that Ba^{2+} carries the regenerative current more readily than Ca^{2+}.

There have been suggestions of cation antagonism in ionic stimulation of ciliary reversal in *Paramecium* (Jahn, 1962; Naitoh, 1968) somewhat resembling the Ca-Na antagonism seen in cardiac muscle (Lüttgau & Niedergerke, 1958). On the surface there appears to be an antagonism between [K] and [Ca] affecting the amplitude of the response to depolarizing current (Fig. 10). Inspection of Figs. 7 and 8 shows that the antagonistic effects of Ca^{2+} and K^+ on the amplitude (difference between overshoot and resting potential) result in large part from the predominance of $[Ca]_0$ in determining the resting potential. The result is an increase in base-to-peak amplitude with increasing $[Ca]_0$ and a decrease in amplitude with increasing $[K]_0$. The relation is such that in the higher ranges of $[Ca]_0$ and the lower ranges of $[K]_0$ the amplitudes are more closely related to $[K]/[Ca]^{\frac{1}{2}}$ than to ionic strength (Fig. 10B).

The early repolarizing phase of the action potential could result from either a time-dependent calcium inactivation (reduction in calcium conductance), delayed rectification for potassium, or both. Since the initial component of repolarization is more rapid than is consistent with the time constant of the inactive membrane, delayed rectification appears to play a role in repolarization. The initial rapid down stroke during repolarization is slowed by addition of some cations (K^+, Rb^+, Cs^+), but remains largely unaffected by others (Li^+ and NH_4^+) (Figs. 11 and 12). The increase in overshoot produced by the first group (K^+, Rb^+, Cs^+) may result from an increase in conductance of the membrane toward those ions during excitation and repolarization. As the extracellular concentration of any of these is raised, the net outward current producing repolarization through that conductance is reduced because of the increased inwardly directed electrochemical gradient for those ions. TEA slows the repolarizing phase consistent with its ability to interfere with delayed potassium activation (Hagiwara & Saito, 1959), and provides further evidence that repolarization is active.

The regenerative calcium response in *Paramecium* appears to perform two functions. First, it amplifies the depolarizing receptor potential which spreads electrotonically from the region of transducer membrane at the anterior surface of the cell (Naitoh & Eckert, 1969*a*; Eckert *et al.* 1972). The final depolarization is graded since the receptor current and regenerative response are both graded. Secondly, the increased calcium permeability evoked by the electrotonic spread of depolarizing receptor current leads to a distributed influx of Ca^{2+} which activates the mechanism for ciliary reversal (Eckert, 1972; Eckert & Naitoh, 1972).

SUMMARY

1. Standard intracellular current-passing and recording techniques were used to investigate the electrical properties of the membrane of *Paramecium caudatum*.

2. The surface membrane produces regenerative depolarizations graded in amplitude and rate of rise with the intensity of applied outward current pulses. In a solution of 1 mM-$CaCl_2$, 1 mM-KCl and 1 mM Tris-HCl at pH 7·2 and 18 °C the overshoot reaches a maximum amplitude of +5 to +15 mV, and shows a maximum rate of rise of about 7 V/sec.

3. The overshoot is insensitive to Li^+, Na^+, Cl^-, TTX and procaine, but increases slightly (up to 10 mV/10-fold rise in ion concentration) in the presence of K^+, Rb^+, Cs^+, NH_4^+, Mg^{2+} and tetraethylammonium.

4. The overshoot increases 22–25 mV for each 10-fold increase in external [Ca]. Ba^{2+} and Sr^{2+} convert the graded response to one that is all-or-none.

5. Repolarization following the peak of the response is more rapid than expected from the time constant of the resting membrane, suggesting delayed rectification.

6. A conditioning depolarization causes inactivation of the regenerative response, and hyperpolarization depresses the response to a given stimulus current. Refractoriness persists for up to 200 msec after each response.

7. The regenerative response of the membrane is consistent with a transient increase in conductance to Ca^{2+} (Ba^{2+}, Sr^{2+}) with a consequent inwardly directed calcium current. This agrees with recent evidence that ciliary reversal (which accompanies depolarization) is produced by an influx of Ca^{2+}.

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