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CALCIUM-DEPENDENT REPOLARIZATION IN *PARAMECIUM*

BY PAUL BREHM, KATHLEEN DUNLAP* AND ROGER ECKERT

From the Department of Biology and Brain Research Institute, University of California, Los Angeles, California, 90024, U.S.A.

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SUMMARY

1. Intracellular injection, recording and current-passing methods were used to investigate the role of intracellular Ca in the modulation of electrical behaviour in the ciliate *Paramecium caudatum*.

2. Injection of EGTA converted graded regenerative responses ascribed to Ca inward current to all-or-none action potentials. The EGTA injection also caused a discontinuity in the steady state *I–V* relations to outward current, but had little effect on hyperpolarizing current–voltage responses.

3. The overshoot of the all-or-none spike produced by the EGTA-injected cell followed an approximate 29 mV increase for a tenfold increase in external Ca concentration and was independent of changes in external K and Na concentrations.

4. The EGTA-induced all-or-none action potential tended to produce plateaus that could last up to 20 sec. During the plateau the membrane slowly repolarized to a critical potential, upon which repolarization occurred precipitously.

5. Injection of 10^{-6} M-free Ca^{2+} as a Ca-EGTA buffer hyperpolarized the membrane and decreased the potential shifts to inward current pulses. These responses are consistent with an increase in K conductance.

6. During EGTA plateaus reversed beating of the cilia indicated a rise in intracellular Ca, and thus an inability of the EGTA to complex the Ca as rapidly as it entered the cilia. Reversal of the motile apparatus thus appears to be activated at lower concentrations of intracellular Ca than are required to activate the inferred Ca-dependent K system.

7. In uninjected cells removal of the cilia, which results in a loss of the voltage-activated Ca channels (Dunlap, 1977), or addition of extracellular Ba both tended to linearize the steady state *I–V* relations.

8. Injections of Cs and TEA tended to linearize the steady state *I–V* relations, but did not result in either a conversion to an all-or-none spike or a discontinuity in the depolarizing steady-state *I–V* relations.

9. It is concluded that in *Paramecium* a Ca-activated K conductance short-circuits the inward current of the regenerative Ca response, preventing all-or-none behaviour. The occurrence of plateau spikes following EGTA injection indicates that the Ca conductance inactivates very slowly in face of a maintained depolarization. Such

* Present Address: Department of Pharmacology, Harvard Medical School, Boston, Massachusetts, 02115.

slow Ca-inactivation is consistent with the slow relaxation of Ca-dependent ciliary reversal that occurs during maintained depolarization.

10. The possibility is discussed that injection of EGTA may also enhance the Ca conductance.

INTRODUCTION

An inward Ca current is responsible for the regenerative depolarization produced by the ciliate *Paramecium* (Naitoh & Eckert, 1968; Kung & Eckert, 1972; Naitoh, Eckert, & Friedman, 1972). The response is graded with stimulus intensity, and the relationship between spike overshoot and Ca concentration is less than the predicted +29 mV increment for a tenfold increase in Ca concentration (Naitoh *et al.* 1972). Addition of Ba to the recording solution results in an all-or-none spike which fits the predicted Nernst slope for altered external Ba (Naitoh & Eckert, 1968). These findings led to the proposal that the inward current in *Paramecium* is short-circuited by a K conductance that is activated by entry of Ca some time before the peak inward current is achieved (Eckert, Naitoh & Machemer, 1976). A Ca dependency of certain K conductances has already been inferred in other tissues (Whittam, 1968; Meech & Strumwasser, 1970; Krnjević & Lisiewicz, 1972; Meech & Standen, 1975; Clusin, Spray & Bennett, 1975; Isenberg, 1975*a*, *b*; Heyer & Lux, 1976).

We have used both injected Ca chelator, EGTA, and injected Ca to examine the role of inward Ca current and intracellular Ca^{2+} in the activation of a K conductance in *Paramecium*. The graded response was converted to an all-or-none action potential with a plateau of variable duration under conditions where the elevation of internal Ca levels was limited by injected EGTA. Conversely, experimental elevation of intracellular Ca led to an activation of a repolarizing conductance. These results suggest that a component of K conductance is regulated by Ca ions entering the cell during depolarization. The results are also consistent with a possible additional action of intracellular Ca, namely a modulation of the membrane Ca conductance.

METHODS

Specimens of *Paramecium caudatum* were reared on hay infusion and were isolated for electrophysiological investigation by methods described elsewhere (Naitoh & Eckert, 1972). Recording and stimulating electrodes had tip diameters of less than 0·2 μm and when filled with 1 M-KCl they had resistances between 80 and 150 MΩ in normal recording solution. A bevelled pipette with a 1·0–2·0 μm diameter at the base of the bevel was used to inject solutions into the cell. The solutions were injected into the cell with pressure pulses of 1–2 sec duration at 1 lb./in.^{2} pressure. Throughout this procedure the flow of solution from the 2 μm injector tip into the cytoplasm was visually monitored with the aid of a 40 × water-immersion Zeiss phase objective. During the entire injection procedure the electrical properties of the cell were monitored. Specimens were discarded if the resting membrane depolarized irreversibly or if the input resistance fell below 20 MΩ. Experiments were carried out at temperatures of 18–20 °C.

The normal bath solution (Ca solution) contained 1 mM-$CaCl_2$, 4 mM-KCl, 0·1 mM-EDTA (ethylenediamine-tetraacetic acid), and 1 mM-Tris-base adjusted to pH 7·2. The EDTA (which was normally added to chelate deleterious traces of heavy metal, and thereby enhanced the viability of impaled cells) was excluded from the solution in experiments where the Ca Nernst plot was determined. Also, in some experiments 4 mM-$BaCl_2$ was added to the Ca solution. This solution will be termed the Ba solution. The following solutions were pressure injected into cells; 20 mM-K-EGTA (ethylene-glycol-bis-aminoethylether-*N*,*N*-tetraacetic acid), 20 mM-KCl, 100 mM-TEA Cl (tetraethylammonium chloride), 100 mM-CsCl, and pCa buffers made up from 20 mM-$CaCl_2$ plus sufficient K-EGTA to yield free Ca concentrations of 10^{-6} M (Portzehl, Caldwell

& Rüegg, 1964). All of the internally injected solutions contained 20 mM-Pipes (piperazine-*N*,*N*-bis-2 ethane sulphonic acid) buffer at pH 7·0.

To estimate the amount of solution displaced from an injection pipette, bevelled injectors were back-filled with [^{3}H]thymidine. The isotope was then injected with a 1 sec pulse into a scintillation vial. The counts per minute were compared with a known standard for an estimate of the amount of solution ejected. The results indicate that $2–6 \times 10^{-11}$ l. exit the electrode with a single 1 sec pulse. This corresponds to approximately 1/10–1/30 of the cell volume (Iida, 1940) per injection. In the case of CsCl and TEA-Cl as many as ten injections were made on a single cell, representing a volume of injected solution approximately equal to the volume of the cell. Since the cells did not undergo corresponding increases in volume other than a transient minor swelling after each injection pulse, it appears that the water contained in the injected solutions was removed from the cell either across the surface membrane or via the contractile vacuole. Injected cells continued to give a healthy appearance both morphologically and in electrical properties with all solutions except in the case of multiple Ca injections. After a few Ca injections the membrane depolarized and the input resistance fell irreversibly.

Experiments with Ca-blocking agents such as Mn^{2+}, Co^{2+}, and La^{3+} or without extracellular Ca were not carried out, since these procedures have either complex or deleterious effects on the cell (Naitoh *et al.* 1972; Naitoh & Eckert, 1972; Friedman & Eckert, 1973).

RESULTS

I Effects of injected EGTA

Paramecium normally generates a graded regenerative response ascribed to a calcium inward current (Eckert *et al.* 1976) to applied outward currents of increasing intensity (Fig. 1). Only with large currents does the response exhibit little further increase in amplitude. Injection of EGTA in an amount producing an intracellular concentration of approximately 1 mM transformed the graded response into an all-or-none spike (Fig. 1). The threshold of the spike was only about +3 mV from the resting potential, and the spike amplitude (i.e. resting potential to peak) in normal Ca-solution was 30–35 mV with an overshoot of 0 to +10 mV. Further injections of EGTA did not result in any further increase in spike amplitude or change in threshold. The injection of the EGTA caused a depolarization of 0–10 mV. However, within a few minutes the membrane potential returned to the original resting value which averaged −25 mV in normal Ca-solution. Multiple injections of EGTA generally resulted in a permanent decrease in both the resting membrane resistance and in the slope resistance to inward current pulses (Fig. 1). Injections of 20 mM-KCl with 20 mM-Pipes buffer containing no EGTA resulted in a similar depolarization and decrease in both the resting and slope resistance. Therefore, the decrease in resistance with EGTA was most probably due to a non-specific effect of the injection rather than a specific effect of the EGTA. In any event, it rules out a simple drop in leakage conductance as the cause of the findings that follow.

The injection of EGTA causes a discontinuity in the *I–V* plot to outward current of as little as 10^{-10} A (Fig. 1). The corresponding jump in the steady state potential from −25 to 0 mV was always preceded by the upstroke of an all-or-none action potential exhibiting an overshoot 5–10 mV higher than the subsequent plateau (Fig. 1).

The overshoot of the all-or-none spike of the EGTA-injected cell was a function of the extracellular Ca concentration and was independent of K and Na (Fig. 2). The overshoot followed a 29 mV increase per tenfold increase in the external Ca concentration over the range 0·1–5·0 mM, which agrees with the predicted behaviour

of a pure Ca spike. The slope deviated from 29 mV between 0·1 and nominal 0·01 mM, which may simply be due to contaminant Ca or other ionic currents which may become significant at low extracellular Ca concentrations. At Ca concentrations below 0·01 mM (even though divalent cations were substituted for Ca) viability of the preparation was severely impaired, so that these concentrations could not be tested.

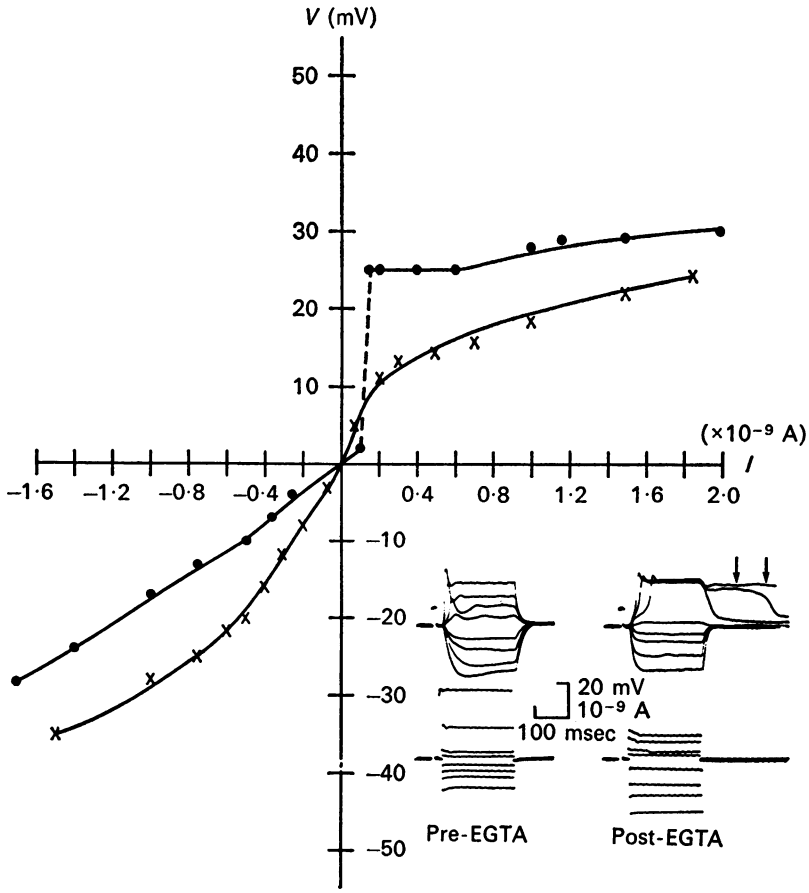

Fig. 1. *I–V* relations in a cell before (X) and after (●) EGTA injection in a cell bathed in the normal Ca solution. Steady-state voltages were measured at the end of a 200 msec stimulus pulse. The origin of the *I–V* plot represents the resting potential, which in this case was −25 mV. The oscilloscope records are those from which the *I–V* relations were taken. Upper trace, voltage; lower trace, stimulus current. The arrows on the records point out a plateau that is seen following termination of stimulus current.

Injection of EGTA converted the graded depolarizations evoked by outward stimulating current to all-or-none action potentials (Fig. 3). The maximum rate of rise of the upstroke, and hence the net inward current was also increased by EGTA injection. The all-or-none action potentials frequently exhibited a plateau (Figs. 1, 4 and 6), having an absolute potential of about 0 mV. Over a period of up to 20 sec the membrane slowly repolarized toward about −5 mV, beyond which repolarization to the resting potential occurred precipitously (Fig. 4).

The plateau would not develop if the cell was stimulated too soon after a previous action potential. For a few minutes following EGTA injection all-or-none spikes with long plateaus occurred spontaneously, but with time the cell ceased to be spontaneous. Spontaneously occurring and stimulus-induced responses appeared to be identical

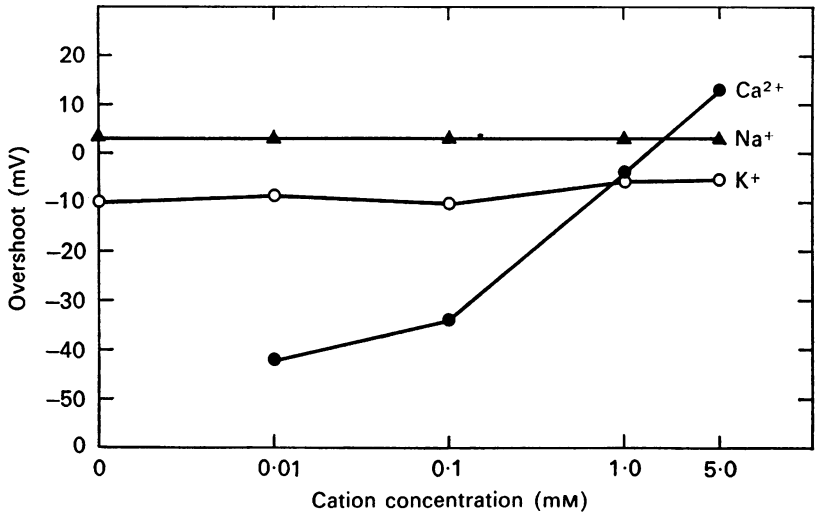

Fig. 2. The overshoot of the spike after EGTA injection recorded in various Ca (●), Na (▲), and K (○) solutions. Ca was held at 1 mM, K at 4 mM, and Na at 0 mM except as indicated. EDTA was not included in the normal solution in this experiment. The absolute membrane potential varied between different cells in identical recording solutions. Therefore, all points for each ion series were measured on one cell.

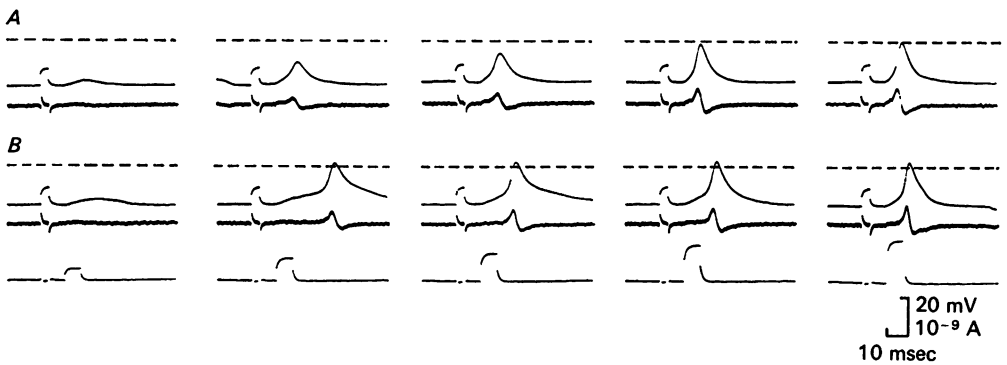

Fig. 3. Responses to 10 msec stimulus pulses prior to EGTA injection, *A*, and after EGTA injection, *B*. The traces in *A* and *B* from top to bottom represent reference potential, membrane potential, first derivative of the membrane potential, and applied current with intensity increasing to the right. The spike exhibits a distinct threshold and all-or-none behaviour only after EGTA injection. *A* 10 mV, 10 msec calibration pulse precedes each membrane response.

(Fig. 4). For a given cell the duration of the plateau remained relatively constant, but between cells the duration differed (Fig. 4). The absolute potential of the plateau, like the spike, was unaffected by changes in external K between 5·0 and 1·0 mM. It

was not possible to examine the effects of a wide range of external K concentrations on plateau amplitudes, as was done with the spike, since the plateau shortened and disappeared at low K concentrations (Fig. 5). Likewise, the plateau was absent in external Ca concentrations below 1·0 mM. Thus it appears that in EGTA-injected cells repolarization is favoured by a reduced inward Ca gradient and an enhanced outward K gradient. The converse applies to plateau production.

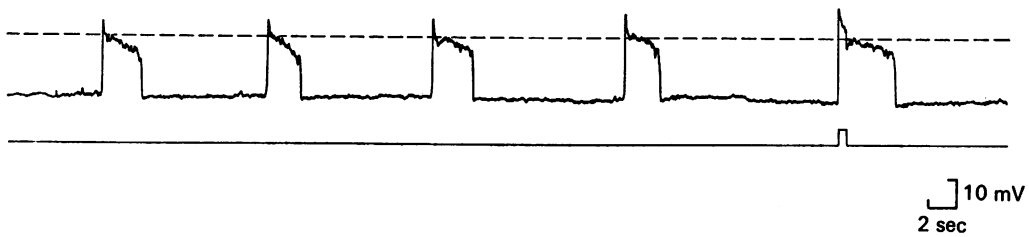

Fig. 4. Prolonged plateaus following EGTA injection. Upper trace, membrane potential; lower trace, applied current. Four spontaneous action potentials lasting approximately 4 sec each are observed. These are followed by an action potential evoked by a depolarizing current pulse.

It can be inferred from two observations that the Ca conductance during the entire plateau was higher than the resting Ca conductance. First, in most preparations the cilia beat in reverse during the entire duration of the plateau. They always reverted back to the normal beating direction immediately upon repolarization. Ciliary reversal requires that the intraciliary Ca concentration be elevated (Naitoh & Kaneko, 1972). The Ca influx that maintains the elevated level must enter through the ciliary membrane, through which the regenerative current enters the cell (Dunlap, 1977). Secondly, the inward current test pulses (Fig. 6*B*) frequently resulted in a repolarization of the membrane potential back to the resting level, but when the inward pulse was turned off, the potential returned to the level of the plateau. This positive potential shift requires a net inward current, and hence a high conductance for an ion that has an equilibrium potential more positive or similar to the plateau potential. In contrast to EGTA-injected cells (Fig. 6*B*), non-injected cells which were depolarized for 10 sec to 0 mV level showed a large total conductance increase which remained high for the duration of the clamp (Fig. 6*A*). Since Ca conductance was not blocked by EGTA injection, the decrease in total conductance which accompanied the injection was probably caused by a depressed K conductance.

To test the possibility that the action of EGTA occurs by means independent of Ca entry, EGTA was injected into deciliated cells, since these have only a residual depolarization-activated Ca conductance (Dunlap, 1977). The results in Fig. 7 demonstrate only a slight decrease in the slope and resting membrane resistance. The residual effect may reside in the oral cilia that are resistant to deciliation. No discontinuity in the $I-V$ plot and no increased excitability resulted from the EGTA injection in deciliated cells (Fig. 7).

Injection of EGTA generally did not block the ciliary reversal which accompanied the Ca spike, and the cilia would remain reversed for the entire duration of a plateau. In every case the cilia immediately returned to the normal beat orientation upon repolarization. In a few EGTA-injected cells, however, ciliary reversal during

the spike or plateau was blocked. That is to say, ciliary reversal, which is dependent on an increase in $[Ca]_i$ (Eckert, 1972), was uncoupled from membrane depolarization, presumably because the injected EGTA in such cases effectively limited $[Ca]_i$ to levels below those required for reversed beating.

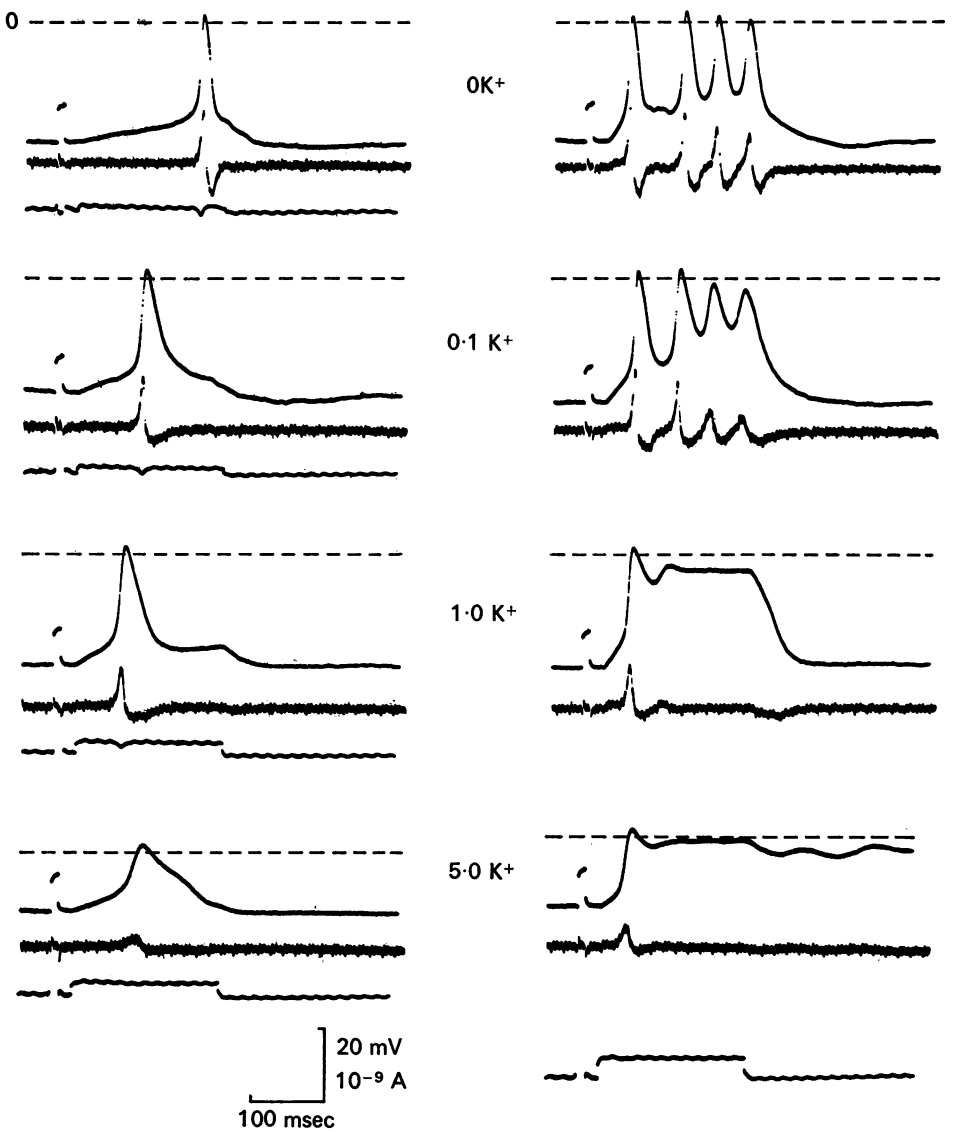

Fig. 5. Effect of changes in external K on evoked action potentials after EGTA injection. The K concentrations (mM) are indicated between columns. The left column shows just-threshold spikes with increasing external K concentrations. Upper to lower traces are reference potential, membrane potential, first derivative of membrane potential, and applied current. The right column shows the response with a stronger stimulus. The applied current for the right column was the same in each case, and is shown by a single sweep at the bottom of the column. Repolarization is slowed in high external K. In both the right and left columns the overshoot is independent of external K concentrations.

Injection of Ca-EGTA buffer solutions containing 10^{-6} M free Ca resulted in a membrane hyperpolarization in ciliated specimens of as much as 10 mV and a concomitant conductance increase (Fig. 8). Following injection of the Ca buffer, the membrane repolarized over the course of approximately 20 sec, at which time the conductance also returned to the original resting value (Fig. 8).

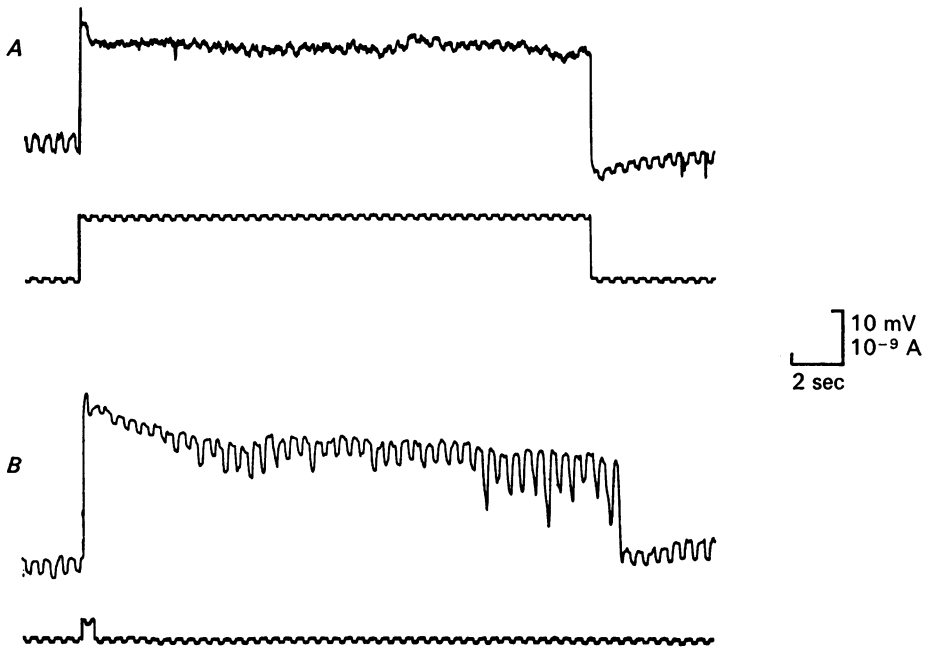

Fig. 6. A comparison of membrane conductance before (A) and after (B) EGTA injection. Upper trace, membrane potential; lower trace, current. Hyperpolarizing pulses were passed to measure the membrane conductance. Note the high membrane conductance throughout the depolarization in the uninjected cell, and the high conductance and large hyperpolarization that follows repolarization. The EGTA-injected cell shows low conductance and slow repolarization during the plateau. Moreover, following repolarization the input conductance is only slightly higher than at rest, and the potential does not undershoot the original resting level.

II K-blocking agents

TEA and Cs were injected into *Paramecium* in amounts sufficient to raise the intracellular concentrations to at least 10 mM. Injections of either TEA or Cs initially depolarized the cell, commensurate with a reduction in resting K conductance, but within several minutes the membrane returned to the original resting level. Both TEA and Cs diminished outward rectification and tended to linearize the *I–V* plot (Fig. 9). However, after some delay (> 3 min) these injected ions had little effect on the resting resistance and the hyperpolarizing slope resistance (Fig. 9). Even with repeated injections of TEA and Cs, the upstroke of the Ca response did not become all-or-none, nor exhibit a plateau (Fig. 9).

TEA appears to block rather selectively the voltage-activated (and not the Ca-activated) K conductances in some cells (Heyer & Lux, 1976; Thompson, 1977).

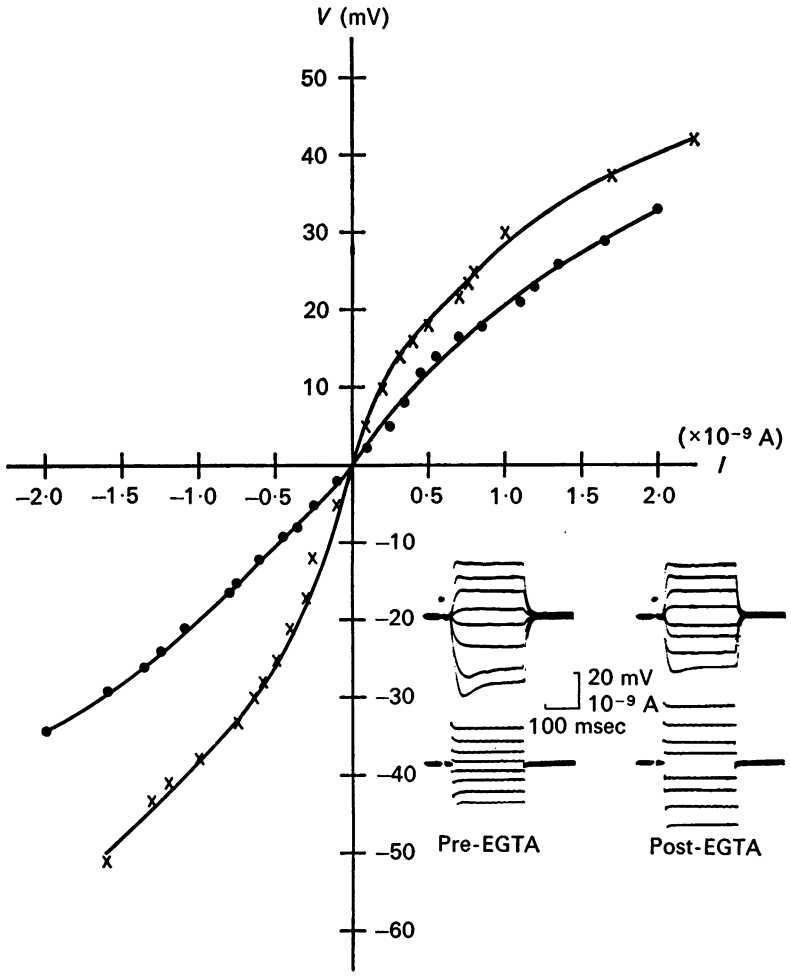

Fig. 7. *I–V* relations in a deciliated cell before (X) and after (●) EGTA injection. Steady-state voltages were measured at the end of a 200 msec pulse. The resting potential was −27 mV. The records are those from which the *I–V* relations were plotted. Upper trace, voltage; lower trace, stimulus current. A small drop in membrane resistance accompanied the EGTA injection. Both the pre- and post-injected responses are non-regenerative.

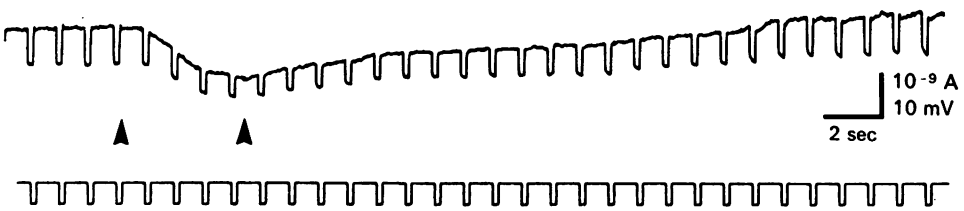

Fig. 8. Response to a single pulse of pressure-injected Ca-EGTA buffer containing 10^{-6} M free Ca. The arrows indicate the beginning and end of the Ca injection. Inward 0·5 nA current pulses 200 msec long were passed throughout the procedure. An immediate hyperpolarization and conductance increase occurred in response to the Ca injection. Recovery took place over approximately 20 sec.

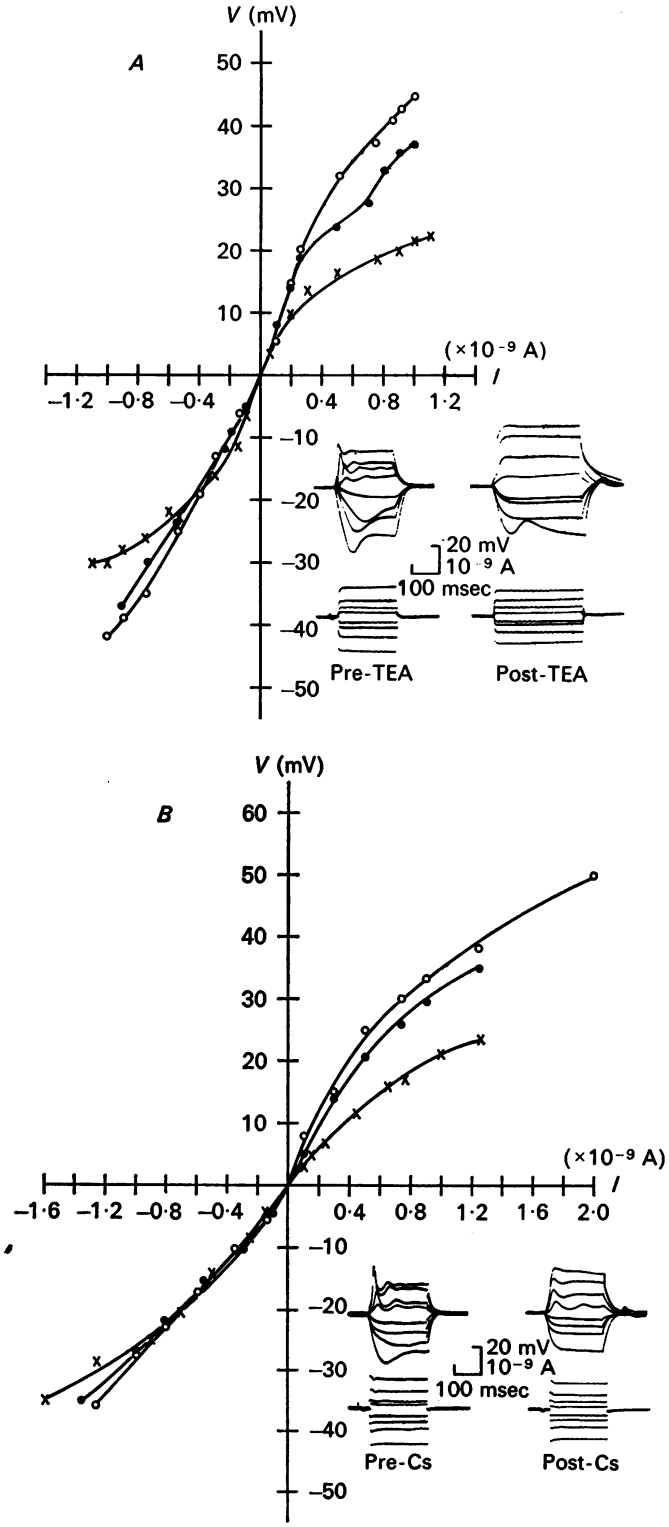

Fig. 9. For legend see facing page.

If this is also true in *Paramecium*, these results would suggest that the Ca-activated K conductance alone is sufficient to short-circuit and limit the Ca response.

III Changes produced by deciliation

Removal of the cilia resulted in a loss of the Ca response (Dunlap, 1977), and a linearization of the *I–V* plot (Fig. 10). The increase in the slope resistance with deciliation is partially due to an increase in input resistance with the loss of about 50 % of the surface membrane area. However, in spite of the increased input resistance, the *I–V* relations showed a significant reduction in outward rectification. This effect was reversed with regrowth of the cilia (Fig. 10). Furthermore, return of the rectification paralleled the time course of the return of the Ca response (not shown).

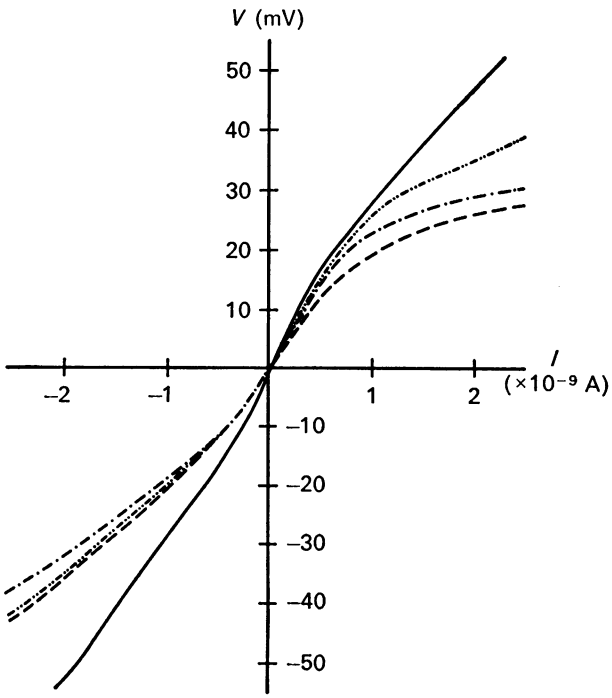

Fig. 10. Steady-state *I–V* relations in a representative control (– – –), deciliated (——), 3 hr reciliated (— . .), and 24 hr reciliated (—.) cell. The curves were generated with a 10 sec current ramp, beginning at rest, first depolarizing and then hyperpolarizing. 5 mM-$MgCl_2$ was included in the bath solution to stabilize the membrane of deciliated cells.

Fig. 9. *A*, steady-state *I–V* relations before (X) and after (○) TEA injection. Filled circles, single injection of 100 mM-TEA: open circles, after many injections. The plot becomes linearized in the depolarizing direction by the TEA injection. The resting potential of the cell was −25 mV. The upper trace, voltage; lower trace, current. Duration of the stimulus was increased from 200 to 300 msec after TEA injection in order to reach a steady-state voltage. *B*, steady-state *I–V* relations before (X) and after (○) Cs injection. Filled circles, single injection of 100 mM-Cs; open circles, after many injections. The resting potential was −25 mV. The accompanying traces were taken from the cell from which the *I–V* plot was made. Upper trace, voltage; lower trace, current.

Linearization of the *I–V* relations produced by removal of the cilia (Fig. 10), can be explained by the localization of the Ca channels in the cilia (Dunlap, 1977). Ca activation of a K conductance would therefore be eliminated by deciliation, diminishing rectification. Mutant *Paramecium aurelia* cells that have an impaired Ca conductance (Kung & Eckert, 1972; Satow & Kung, 1976; Schein, Bennett & Katz, 1976) show considerable rectification to outward current pulses, which may indicate that the voltage-activated K channels as well as the Ca and Ca-activated K channels are largely restricted to the membrane covering the cilia.

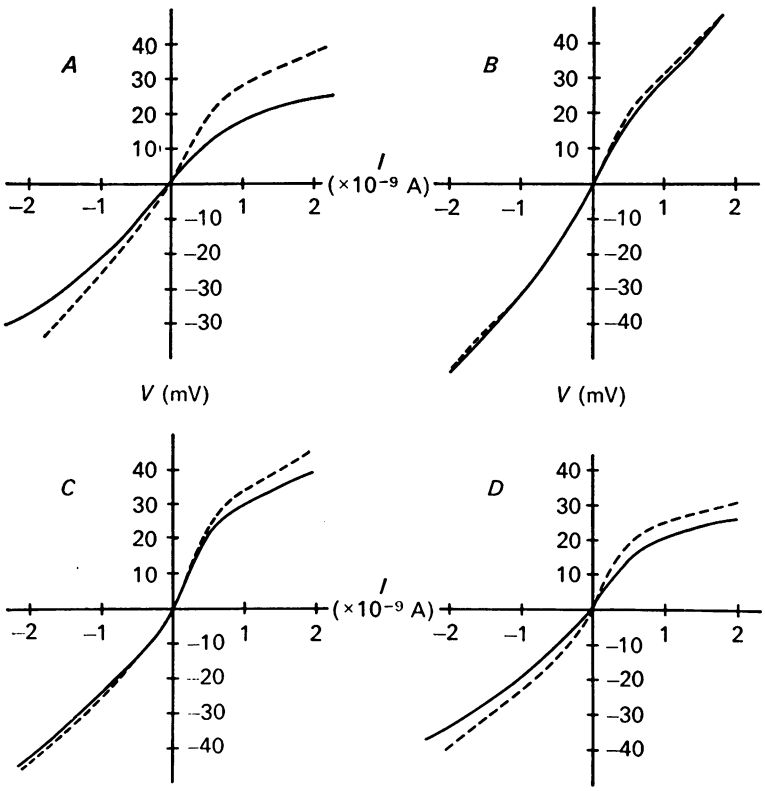

Fig. 11. *I–V* relations in a representative (*A*) control, (*B*) deciliated, (*C*) 3 hr reciliated, and (*D*) 24 hr reciliated cell in control (continuous lines) and 4 mM-Ba (dashed lines) solutions. The curves were generated in the same manner as those in Fig. 10.

IV Action of extracellular Ba

Addition of 4 mM-Ba to the Ca solution of an otherwise untreated *Paramecium* converted the graded response to an all-or-none spike (Naitoh & Eckert, 1968). If Ba suppressed the K conductance resulting in all-or-none behaviour, Ba should also linearize the *I–V* plot. However, the cells spiked repetitively in the presence of Ba, making steady-state voltages difficult to attain. A 10 sec current ramp was used to allow the membrane time to accommodate and approach a steady state. Ba linearized the *I–V* plot similar to the changes observed with deciliation (Fig. 11). Addition of Ba to deciliated cells lacking all but a residual Ca conductance (Dunlap, 1977)

resulted in no change in the *I–V* relations (Fig. 11*B*). As the cilia regrew, however, the effects of Ba on the steady-state *I–V* relations returned to normal (Fig. 11*C*, *D*).

The action of Ba in producing all-or-none behaviour in *Paramecium* (Kinosita, Dryl & Naitoh, 1964; Naitoh & Eckert, 1968) suggests that Ba, although transversing the Ca channels, is less effective than Ca in activating a K conductance. The transformation to an all-or-none spike, the prolongation of the spike, and the increased amplitude of the spike in *Paramecium* seen in the presence of Ba (Naitoh & Eckert, 1968; Eckert *et al.* 1976) are consistent with reduction in repolarizing outward current, as is the partial linearization of the *I–V* plot by Ba. In addition, Ba may have greater mobility in the Ca channel than Ca itself (Hagiwara, Fukuda & Eaton, 1974) and thus carries a stronger inward current than Ca.

DISCUSSION

Graded Ca responses in *Paramecium* (Naitoh *et al.* 1972; Eckert *et al.* 1976) were transformed into all-or-none spikes by the injection of EGTA that decreased the internal free Ca to 10^{-8} M or below. Injection of EGTA into cells lacking the depolarization-activated Ca conductance because they were deciliated (Dunlap, 1977) exhibited no change in electrical properties in response to such injections. The simplest explanation would appear to be that the effects of injected EGTA stem from a restriction of the rise in the concentration of internal Ca which normally results from the inward Ca current through channels located in the cilia. However, unknown pharmacological actions of EGTA cannot be ruled out. The results of Ca-EGTA injection experiments where the internal Ca concentration was elevated to 10^{-6} M demonstrate that a rise in intracellular Ca increases the membrane conductance to an ion or ions whose equilibrium potential is more negative than -35 mV (Fig. 8). Since removal of Cl ions has no effect on the electrical responses of *Paramecium* (Naitoh *et al.* 1972), the most likely candidate for the Ca-activated conductance is K, which has an equilibrium potential of about -37 mV with 4 mM extracellular K (Naitoh & Eckert, 1973). Other evidence that a rise in intracellular Ca in *Paramecium* turns on a K conductance is discussed elsewhere (Eckert *et al.* 1976).

The activity of the cilia provide an independent means of estimating changes in intraciliary Ca activity. The intracellular free Ca concentration reaches 10^{-6} M when ciliary reversal occurs in the cell (Naitoh & Kaneko, 1972). Since reversal always accompanies the Ca response, it is reasonable to assume that the intracellular Ca concentration during a Ca response transiently reaches or exceeds 10^{-6} M. This is in agreement with the calculated Ca influx during excitation (Eckert, 1972). Injections resulting in 10^{-6} M free internal Ca result in a hyperpolarization and conductance increase (Fig. 8) as well as ciliary reversal (Saiki & Hiramoto, 1975). It follows that a Ca response is accompanied by an increased repolarizing conductance that is activated by the Ca influx. The increase in the Nernst slope of the Ca response from 25 to 29 mV per tenfold rise in external Ca concentration following EGTA injection, and a change from graded to all-or-none behaviour of the Ca response, indicate that the depolarizing Ca current and the outward repolarizing K current overlap somewhat in time. The Ca-activated K conductance thus would appear to be functional by the time the normal Ca response reaches its peak (Brehm & Eckert, 1977).

It has been inferred from the slow relaxation of reversed beating of the cilia that the Ca conductance in *Paramecium* inactivates very slowly over many seconds (Machemer & Eckert, 1975). A very slow inactivation of the Ca conductance is also indicated by the prolongation of the Ca response into a plateau that persists for up to 20 sec (Fig. 4) after EGTA injection. Under normal conditions the Ca conductance turned on by depolarization appears to be turned off by membrane repolarization resulting from the activation of K conductances. The plateau of the prolonged action potential occurs at the same level as the jump in the late *I–V* relations, suggesting that similar conductance changes are responsible. The repolarization from the plateau following inward and outward pulses cannot be due primarily to the activation of a voltage-activated K conductance, since the membrane potential in EGTA-injected cells exhibits little undershoot or elevated conductance upon repolarization. Furthermore, late in the EGTA-induced plateau, the membrane response to inward current pulses appears regenerative. Such behaviour can result from the inactivation, during a gradual repolarization, of a persisting potential-dependent depolarizing conductance.

Since reversed beating was frequently seen during prolonged plateau spikes after an EGTA injection, it follows that in those cases the EGTA did not prevent some increase in $[Ca]_i$ above its resting level in the confined volume of the cilium. The coexistence of reversed beating and EGTA-induced plateau action potentials result from one or both of the following.

1. The activation of Ca-dependent K conductance may require a greater increase in $[Ca]_i$ than does reversed beating, which appears to require $\geqslant 10^{-6}$ M-Ca^{+2}/l. of intraciliary fluid (Naitoh & Kaneko, 1972). This would permit injected EGTA to diminish K activation and thereby produce plateau spikes without eliminating ciliary reversal.

2. The Ca conductance may be depressed by the intracellular accumulation of free Ca^{2+}. In that case injected EGTA may enhance the Ca conductance and thus the rate of Ca entry, promoting depolarization, and delaying repolarization. An enhanced rate of rise of the upstroke (Fig. 3*B*) suggests an enhanced rate of Ca entry. If such a modulating action of $[Ca]_i$ on the Ca conductance is borne out, it might explain the failure of Oertel, Shein & Kung (1977) to demonstrate under voltage clamp a substantial, sustained Ca current in non-injected paramecia (Eckert, 1977).

Since ciliary reversal was blocked by the injection of EGTA in some cells, intracellular EGTA, under optimal conditions is able to limit the internal rise in pCa sufficiently to uncouple the ciliary responses from membrane depolarization.

The findings provide evidence that accumulation of Ca during depolarization promotes changes in conductance that interfere with all-or-none behaviour and leads to rapid repolarization. A Ca-activated K conductance similar to Ca-dependent K conductances that occur in metazoan cells apparently acts to partially short-circuit the regenerative inward Ca current, normally preventing all-or-none depolarization in this protozoan. The additional possibility, suggested by the persistence of ciliary reversal during EGTA plateaus, that the introduction of the chelator may enhance the calcium current, deserves further exploration.

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Note added in proof. A Ca-activated K conductance was recently reported to occur in *P. aurelia* (Satow, Y., 1977, *Society for Neuroscience*, **3**, 189).

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