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INTERACTIONS OF MEMBRANE POTENTIAL AND CATIONS IN REGULATION OF CILIARY ACTIVITY IN *PARAMECIUM*

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SUMMARY

Ciliary activity in *Paramecium* was investigated in different external solutions using techniques of voltage clamp and high frequency cinematography.

An increase in the external concentration of K, Ca or Mg ions decreased the resting potential. It had no effect on ciliary activity.

When the membrane potential was fixed, an increase in external Ca or Mg and, to a lesser extent, an increase in K concentration, raised the frequency of normal beating or decreased the frequency of reversed beating of the cilia. Similar effects resulted from membrane hyperpolarization with constant ionic conditions.

Increase in concentration of Ca, but not of Mg or K, enhanced hyperpolarization-induced augmentation of ciliary frequency. Increase in Ca concentration also specifically augmented the delayed increase in inward current during rapid hyperpolarizing clamp.

The results support the view that $[Ca]_i$ regulates the frequency and direction of ciliary beating. It is suggested that the insensitivity of the ciliary motor system to elevations of the external concentrations of ions results from compensation of their effects on $[Ca]_i$. Depolarization itself appears to increase $[Ca]_i$ while elevation of the external ion concentrations at a fixed membrane potential appears to decrease $[Ca]_i$.

INTRODUCTION

In freshwater ciliate Protozoa, motor responses which are evoked by abrupt changes in the external concentrations of monovalent alkaline ions subside after some time of exposure to the new solution. The duration of potassium-induced backward swimming of *Paramecium* – with the ciliary beat orientation reversed towards the anterior end of the cell – increases with the ratio of $[K]/[Ca]^{\frac{1}{2}}$ in the external medium. It has been suggested, therefore, that ion antagonism may play a role in the regulation of ciliary activity (Jahn, 1962; Naitoh, 1968). Investigations of the effects upon membrane electrical properties of external application of various

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cations have shown that current-voltage characteristics and electrogenic properties of *Paramecium* are correlated with the $[K]/[Ca]^{\frac{1}{2}}$ ratio (Naitoh & Eckert, 1968). Further electrophysiological studies, on the other hand, have given increasing evidence that reversal of the direction of ciliary beating in *Paramecium* is coupled to regenerative membrane depolarization through inward Ca fluxes which raise the intracellular Ca concentration (reviews by Eckert, 1972; Naitoh, 1974; Naitoh & Eckert, 1974; Eckert, Naitoh & Machemer, 1976). Thus, the ciliary motor response is regulated by a conductance limited calcium influx which may be independent of the binding and release of Ca via a cation exchange system.

The present experiments, employing electrophysiological techniques combined with cinematographic recording of ciliary movement, were directed at an elucidation of the action of K, Ca and Mg on membrane properties and ciliary activity in *Paramecium*.

MATERIALS AND METHODS

Paramecium caudatum was reared in hay infusion, was geotactically collected in a solution of 0·1 mM-$CaCl_2$, 1 mM-KCl and 1 mM-Tris-HCl (pH 7·2), and was transferred to another volume of the same solution. For intracellular recording, glass capillary microelectrodes were employed. Electrodes had tip diameters below 0·5 μm, were filled with 1 M-KCl, and had resistances of about 100 megohms. The external voltage electrode, positioned close to the membrane (Fig. 1), had a somewhat larger tip diameter, resulting in a resistance of about 60 megohms with 1 M-KCl filling. The electrophysiological procedure and voltage clamp technique are described elsewhere (Naitoh & Eckert, 1972; Machemer & Eckert, 1975).

Outputs of the recording preamplifiers (Fig. 1) were set to zero in compensation for tip potentials in the starting experimental solution. Recorded values of the *membrane potential* were corrected for errors due to both the difference in tip potentials of the internal voltage electrode before and after insertion, and another small potential apparently generated by coating of the electrode tip with cytoplasm. The procedure was as follows. After withdrawal from the cell, the internal voltage electrode was transferred into a 20 mM-KCl solution connected to the bath with a 3 M-KCl agar bridge. Assuming a cytoplasmic KCl concentration of 20 mM (Yamaguchi, 1963; Naitoh & Eckert, 1973), the difference between the recorded membrane potential and the potential in the 20 mM-KCl solution is the corrected membrane potential. The remaining error will be due to differences in junction potentials between the internal voltage electrode and the 3 M-KCl bridge. This error should be less than 1·8 mV according to the Henderson diffusion equation. Values of the corrected membrane potential that are given in this paper are more negative than conventionally recorded protozoan membrane potentials in highly dilute solutions.

Linear sawtooth ramps of 5·8 s duration were produced with a Wavetek pulse generator (Model 112). The ramps were adjusted to between -12 and $+18$ mV in amplitude, added to a DC base, and fed into a Tektronix 1A7A+132 differential amplifier as command signals (Fig. 1). The slope of the voltage ramp was chosen to produce quasi-steady-state responses of the membrane over a wide range of potentials in a limited period of time.

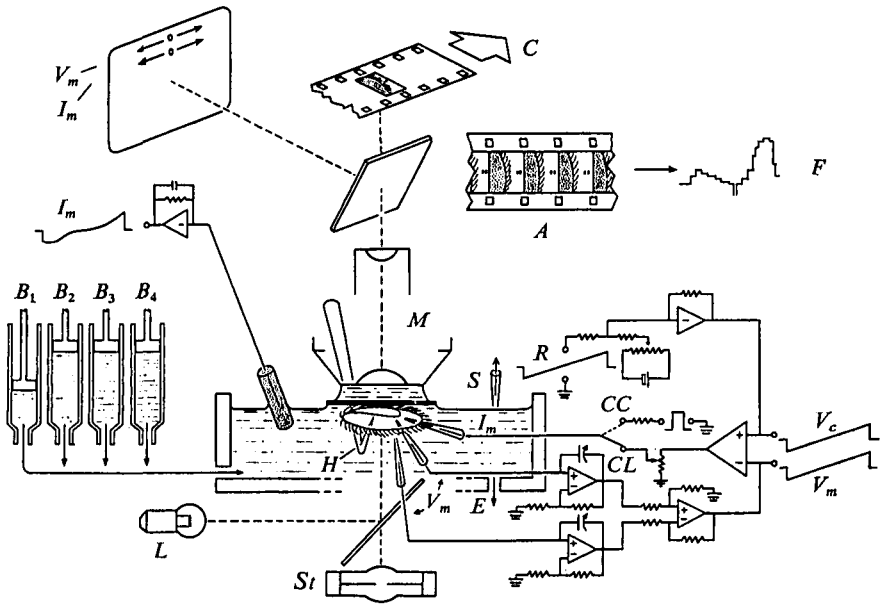

Fig. 1. System of stimulation and recording (see Materials and Methods). *A*, frame analysis of film; B_1–B_4, four solutions of different ionic composition for exchange of bath; *C*, high speed film camera; *CC*, constant current mode; *CL*, clamp mode of stimulation; *E*, evacuation of bath fluid; *F*, resulting frequency diagram; *H*, holding capillary; I_m, membrane current; *L*, pilot lamp; *M*, microscope; *R*, ramp generator; *S*, suction pipette; *St*, strobe lamp; V_c, command voltage; V_m, membrane potential.

The *I/V curves* shown in the figure insets were averaged from data obtained at 1 mV intervals in individual cells. Data points have been omitted for clarity, only joining lines being shown. Membrane resistance was approximated from the initial $\triangle I$ and $\triangle V$ values with a rapid 12 mV hyperpolarization. In this paper, it is termed 'resting resistance' if hyperpolarization occurred from a holding potential equal to resting potential. If the holding potential differed from the resting potential, the term 'resistance at holding potential' is used.

In a series of *experimental solutions* the concentration of one ion, K, Ca or Mg, was increased while other cations were kept constant. Each solution was buffered with 1 mM-Tris-HCl at pH 7·2. The exchange of solutions occurred with rising as well as with decreasing concentrations. It was performed in the unclamped mode, if equilibration toward a new resting potential was desired. The new solution was continuously added to the bath until stabilization of the new potential, which generally required the infusion of 10 bath volumes over a period of 30 s. An additional 150–180 s were allowed after the end of solution exchange before recording.

High speed *cinematography* (Locam 164–5DC) at 200 frames per second was employed to correlate ciliary responses with the membrane events (Fig. 1). Details of the procedure are described elsewhere (Machemer, 1974). Oscilloscope displays of membrane current and voltage were conventionally photographed and also appeared on a second CRT screen, without time base, to be superimposed on the microscopic image of the cilia.

Maintenance of the bath solution at a constant *temperature*, 20 °C, was achieved

with a cooled platform on the miscroscope stage. The temperature was monitored by a thermistor within less than 5 mm of the specimen.

Parameters of the *ciliary response* (frequency and direction of beating) were determined from the 16 mm films by methods described elsewhere (Machemer, 1974). For each filmed cell the frequency of one metachronally coordinated cilium was averaged over three cycles in sequence. If the direction of beating changed, or inactivation of the cilium occurred, before the end of a 3-cycle period, 2 or 4 cycles were averaged in order to correctly represent these changes in the frequency curves. The time course of the frequency and of the direction of beating (orientational response) were recorded graphically, together with the electrical data.

For *processing* of the ciliary responses, data from four different cells, each of which had been exposed to a complete series of concentrations of a particular ion, were computer-averaged by combining two series of *in*creasing concentration with two series of *de*creasing concentration, in order to offset possible effects of the sequence in solutions. Since such effects could not be observed, the averaging procedure of some experiments includes data from a fifth cell. Averaged ciliary beat frequency was plotted at 100 ms intervals by computer, which also recorded beat direction.

RESULTS

1. *Ciliary responses to voltage ramps*

After a stable resting potential was achieved in the experimental solution, the holding potential was adjusted to equal the resting potential. Abrupt hyperpolarization of the membrane increased the frequency of the cilia (+60% in the averaged data shown in Fig. 2*a*). After this initial rise the frequency became a linear function of membrane potential, though oscillations in frequency occurred in individual cilia. When the voltage ramp equalled the holding potential, the frequency was still increased with respect to the prestimulus level (Fig. 2*a*: +20%). This hysteresis of frequency is common during very slow increments in voltage and has been tentatively explained in terms of membrane accommodation (Machemer, 1975). The frequency continued to decrease until a small depolarization was achieved (Fig. 2*a*: +4 mV). With more depolarization the frequency rose again, with the cilia beating in the reversed direction.

Depolarization-induced 'inactivation' of the cilia, i.e. the loss of regular cyclic and directional movements, occurred in the clamped mode and, under certain conditions, also in the unclamped state of the membrane (Machemer, 1974, 1975; Machemer & Eckert, 1975). The strength and the voltage dependence of ciliary inactivation were particularly variable from cell to cell (Fig. 2*b–f*). It should be noted that the true frequency minimum may have been missed as a result of frequency averaging over three sequential cycles. The change in beat direction coincided more or less with the point of minimum frequency. The directional switch was not all-or-none, the degree of change varying with decreasing membrane negativity (Naitoh, 1968), and showing maximal sensitivity at a minimal frequency (Machemer, 1974). A rare example of sideward beating during transition between normal and reversed beating is given in Fig. 2(*f*).

With progressive depolarization above the holding potential the frequency changed

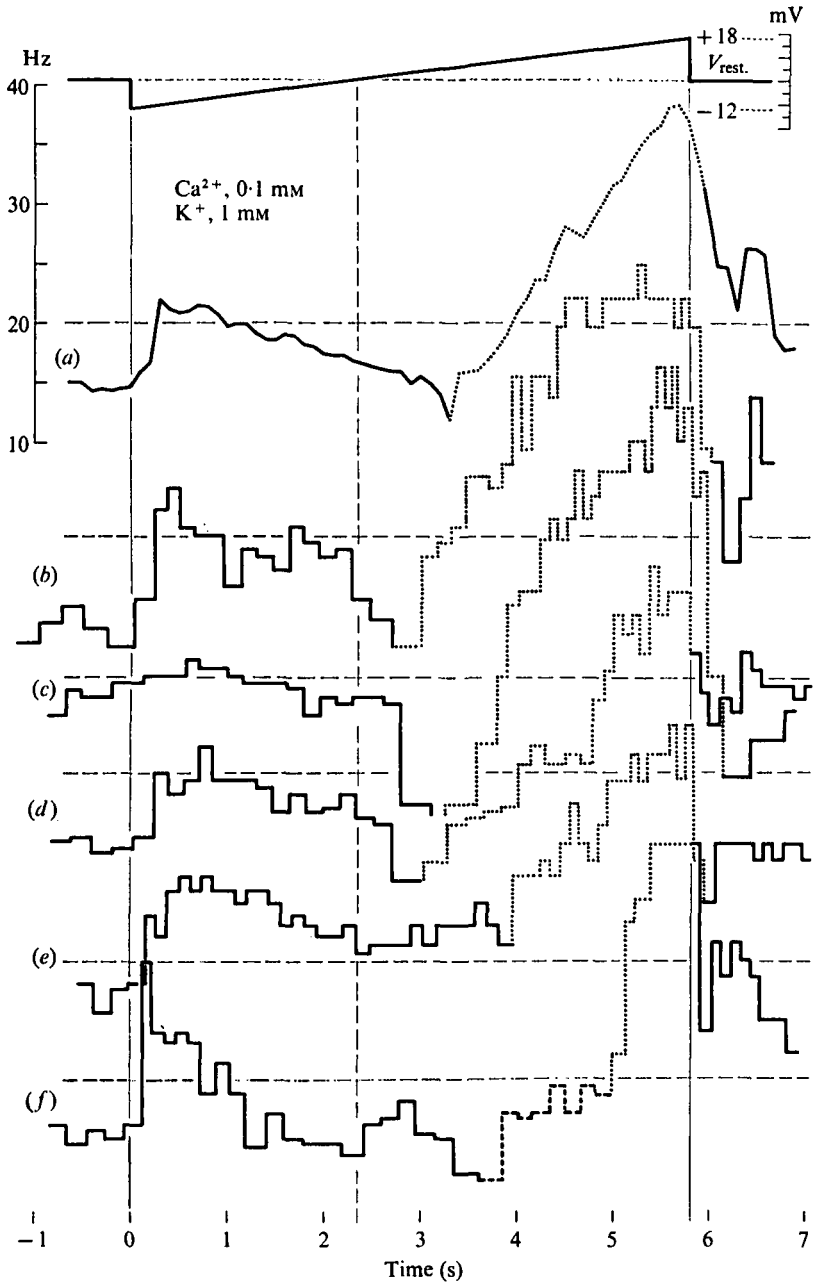

Fig. 2. Motor responses of cilia to voltage-clamped potential ramp. Upper trace shows membrane potential change with time. Holding potential is at the resting membrane potential in each cell. Ramp begins at −12 mV and rises to +18 mV in 5·8 s at approximately 5·2 mV/s. (*b*)–(*f*): frequency responses in 5 individual cells obtained in solution with 0·1 mM-$CaCl_2$ + 1 mM-KCl. Trace (*a*) is computer-generated mean of traces (*b*)–(*f*). Solid lines, normal beat direction; dotted lines, reversed beating; dashed line in (*f*), transient sideward orientation of cilia; interrupted plot between solid and dotted line in (*c*), no frequency measured because cilia were inactivated. Each horizontal section in plot averaged over 3 complete ciliary cycles (occasionally 2 or 4 cycles during periods of transition in activity). Horizontal dashed lines indicate 20 Hz reference level for each plot. Resting potentials (mV): *b*, −60; *c*, −56; *d*, −67; *e*, −69; *f*, −59.

Table 1. *Modifications in* Paramecium *of electrical membrane parameters and ciliary responses to a voltage ramp by equilibration in solutions with varying* [*K*], *and* [*Ca*] *of* 1 *m*M

(Holding potential is equal to the resting potential.)

Solution (mM) [K]	Holding potential (mV)	Resting* resistance (megohm)	Voltage and current at minimal frequency		Frequency (Hz)			
			(mV)	(10^{-10} A)	Pre-stimulus	Maximum hyperpol.	Minimum depol.	Maximum depol.
0·44	−41·9	65	+4·3	+0·4	16	22	11	42
0·95	−43·0	90	+2·8	+0·2	14	23	10	42
1·8	−39·5	69	+3·8	0·0	16	23	5	43
3·2	−38·0	41	+4·9	+0·3	15	23	8	43

* As determined at start of hyperpolarization.

at a rate several times higher than that observed below the holding potential (Fig. 2*a*: 5×). The frequency tended to rise in proportion to the membrane voltage, up to a frequency of 35 Hz. Beyond this frequency the increase appeared to 'level off'. This may have been due to time-dependent Ca inactivation (Machemer & Eckert, 1975). Abrupt repolarization to the initial holding potential was accompanied by a rapid drop in beating frequency and return to the normal beat direction. As a rule, poststimulatory frequency remained at, or rose above, the prestimulus level after passing through a transient minimum. Such 'undershooting' of frequency with rapid repolarization has also been observed in the unclamped cell (Machemer, 1974).

2. *Responses to voltage ramps with holding potential set equal to resting potential with various external concentrations of K, Ca and Mg*

Cells were equilibrated in solutions of various concentrations of K, Ca and Mg. Holding potentials were adjusted to equal the new resting potentials.

2.1. *Change in* [*K*] *with constant* [*Ca*]

Electrical responses. With a Ca level of 1 mM, change of K concentration from 0·44 to 3·2 mM produced a small positive shift in resting potential (Table 1). Resting resistance increased with K concentrations up to 0·95 mM and dropped with higher concentrations, comparable to findings by Naitoh & Eckert (1968). The *I*/*V* curves (inset Fig. 3) were little varied within the K series. A slow negative deflexion of membrane current was seen after the hyperpolarizing start of the potential ramp. This deflexion decreased in size with rising external K concentration.

Ciliary responses. Beat frequency/voltage curves were similar in different K solutions (Fig. 3). The frequency was 14–16 Hz prior to stimulation (Table 1). Ciliary inactivation was pronounced in this series.

2.2. *Change in* [*Ca*] *with constant* [*K*]

Electrical responses. Increasing Ca concentration from 0·1 to 5·1 mM, with 1 mM-K, decreased the resting potential by more than 30 mV (Table 2). Resting resistance showed no substantial variation. During hyperpolarization, inward current rose

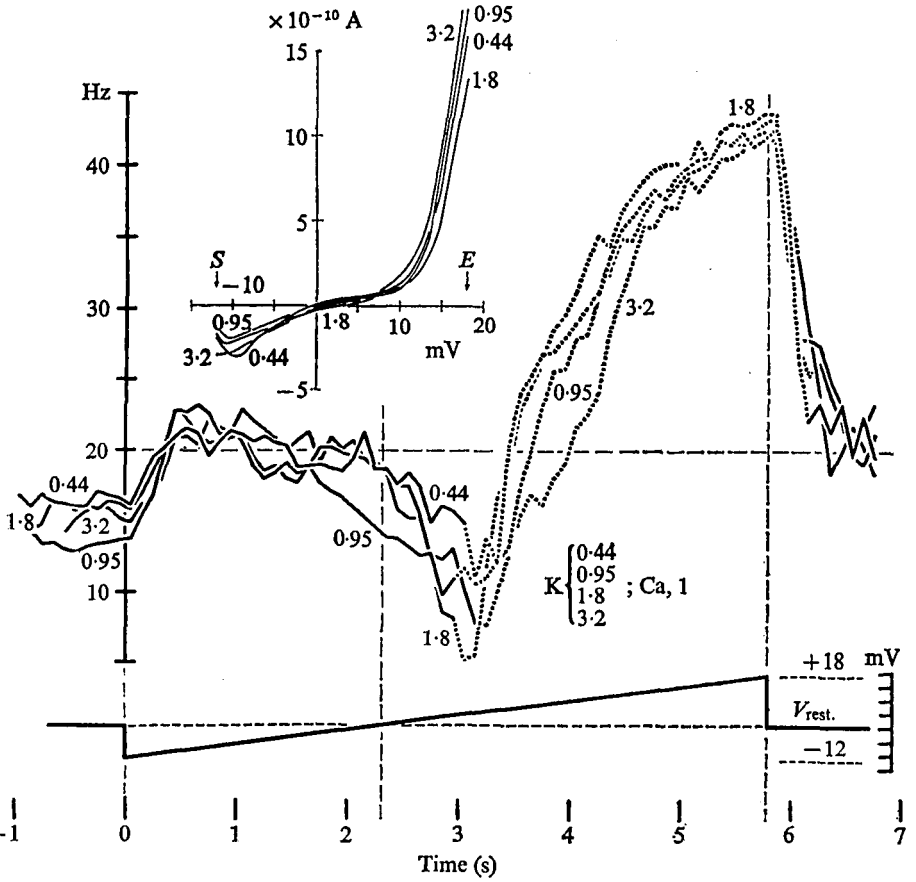

Fig. 3. Potassium series with holding potential equal to resting potential in each solution. Each curve averaged from 4 cells, each of which was exposed to 4 solutions with 1 mM-Ca throughout and K at 0·44, 0·95, 1·8 and 3·2 mM. Mean resting potentials (mV): 0·44 K, −41·9; 0·95 K, −43; 1·8 K, −39·5; 3·2 K, −38. Inset: corresponding averaged *I/V* curves. *S*, start; *E*, end of ramp.

with increased levels of Ca. The slow inward-going membrane current was augmented by Ca (inset Fig. 4).

Ciliary responses. The prestimulus frequency of the equilibrated cells was largely unaffected by variations in external calcium concentration (Table 2). Hyperpolarization-induced augmentation of normal beating, however, increased with Ca up to 1·1 mM. The increase was reduced at 5·1 mM-Ca (Fig. 4). With decreasing negative potential the frequency dropped at a similar slope in all Ca solutions, but the frequency minima were shifted toward less negative potentials with increased Ca concentration.

2.3. *Comparison between frequency/voltage curves obtained in solutions with the same* $[K]/[Ca]^{\frac{1}{2}}$ *ratio*

Ciliary reversal in *Paramecium* can be elicited by the transfer of cells into a solution with an increased $[K]/[Ca]^{\frac{1}{2}}$ ratio. The duration of reversed beating varies in proportion to this increase, leading to the proposal of Ca release from a membrane

Table 2. *Modifications in* Paramecium *of electrical membrane parameters and ciliary responses to a voltage ramp by equilibration in solutions with varying [Ca] and [Mg], and [K] of* 1 mM

(Holding potential is equal to the resting potential.)

Solution (mM) [Ca]	+ [Mg]	Holding potential (mV)	Resting* resistance (megohm)	Voltage and current at minimal frequency (mV)	(10^{-10} A)	Frequency (Hz) Pre-stimulus	Maximum hyperpol.	Δ%	Minimum depol.	Δ%	Maximum depol.	Δ%
0·1†	—	−62·0	56	+5·2	+0·7	15	22		12		38	
0·1‡	—	−63·0	53	+2·7	+0·6	16	18		13		38	
0·3	—	−55·5	53	+6·1	+0·6	17	27	+23	18	+50	41	+8
0·1	0·2	−58·7	56	+2·7	+0·3	15	20	+11	12	−8	36	−5
1·1	—	−44·7	45	+10·1	+2·5	15	32	+46	19	+58	39	+3
0·1	1·0	−44·8	65	+4·9	+0·3	15	20	+11	12	−8	36	−5
5·1	—	−30·3	55	+8·9	+1·1	14	27	+23	17	+42	35	−8
0·1	5·0	−31·8	50	+3·6	+1·1	13	16	−11	9	−31	32	−16

* As determined at start of hyperpolarization.
† Reference for Ca-series.
‡ Reference for Mg-series.

cation-exchange system as the basis of reversed beating (Jahn, 1962; Naitoh & Yasumasu, 1967; Naitoh, 1968). Frequency curves of the K and Ca series (Figs. 3 and 4) were combined to form pairs in such a way that each member of a pair had the same $[K]/[Ca]^{\frac{1}{2}}$ ratio though it was produced with different ionic strengths. As shown in Table 3, the averaged prestimulus frequency did not substantially differ nor show a common tendency between members of pairs or between pairs with falling ratio. Differences between paired frequency/voltage curves of stimulated cells could not be correlated with the modified $[K]/[Ca]^{\frac{1}{2}}$ ratio. Thus, the ratio of concentrations of K and Ca does not appear to significantly influence ciliary activity in the unstimulated and electrically stimulated cell.

2.4. *Change in [Mg] with constant [K] and [Ca]*

Electrical responses. In the presence of 0·1 mM-Ca and 1 mM-K, an increase in Mg concentration from zero to 5 mM decreased the average resting potential by amounts very similar to those produced by an increase of Ca concentrations from 0·1 to 5·1 mM (Table 2). The resting resistance was essentially unaltered. Mg did not increase the activation of net inward current during the hyperpolarizing potential ramp (inset Fig. 5) as did Ca (Fig. 4). No modification of the I/V curve was observed.

Ciliary responses. Mg did not modify the prestimulus frequency (Fig. 5, Table 2). Beat frequency/voltage curves generated by the ramp were not significantly altered by different concentrations of Mg.

2.5. *Comparison of Ca series with Mg series*

Ca and Mg had similar effects on the electrical properties of the resting membrane. During the voltage ramp, however, increased Ca raised the net inward current,

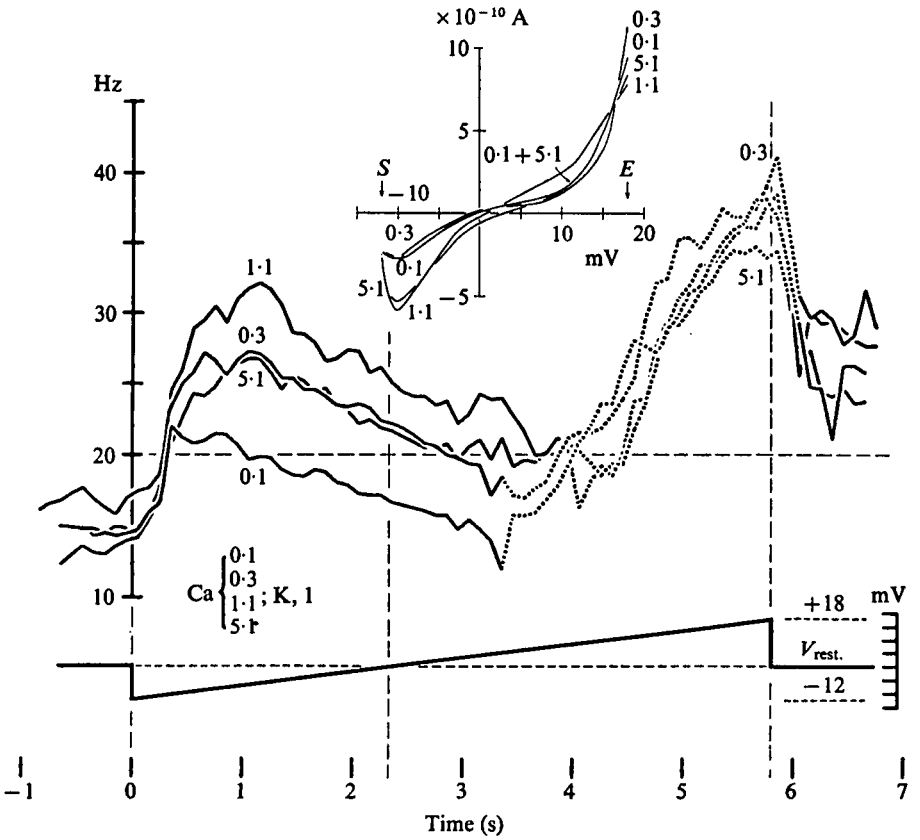

Fig. 4. Calcium series with holding potential equal to resting potential in each solution. Each curve averaged from 5 cells; each cell exposed to 4 solutions with potassium at 1 mM and calcium at 0·1, 0·3, 1·1 and 5·1 mM. Mean resting potentials (mV): 0·1 Ca, −62; 0·3 Ca, −55·5; 1·1 Ca, −44·7; 5·1 Ca, −30·3. Inset: averaged I/V curves under same conditions.

Table 3. *Prestimulus frequencies of cilia obtained in four pairs of experiments, each of which was performed in solutions with the same* $[K]/[Ca]^{\frac{1}{2}}$ *ratio at different ionic strengths*

(Holding potential is equal to the resting potential.)

Solution (mM)				Prestimulus frequency (Hz)
[Ca]	+	[K]	$[K]/[Ca]^{\frac{1}{2}}$	
1		3·2	3·2	15
0·1		1		15
1		1·8	1·8	16
0·3		1		17
1		0·95	0·95	14
1·1		1		15
1		0·44	0·44	16
5·1		1		14

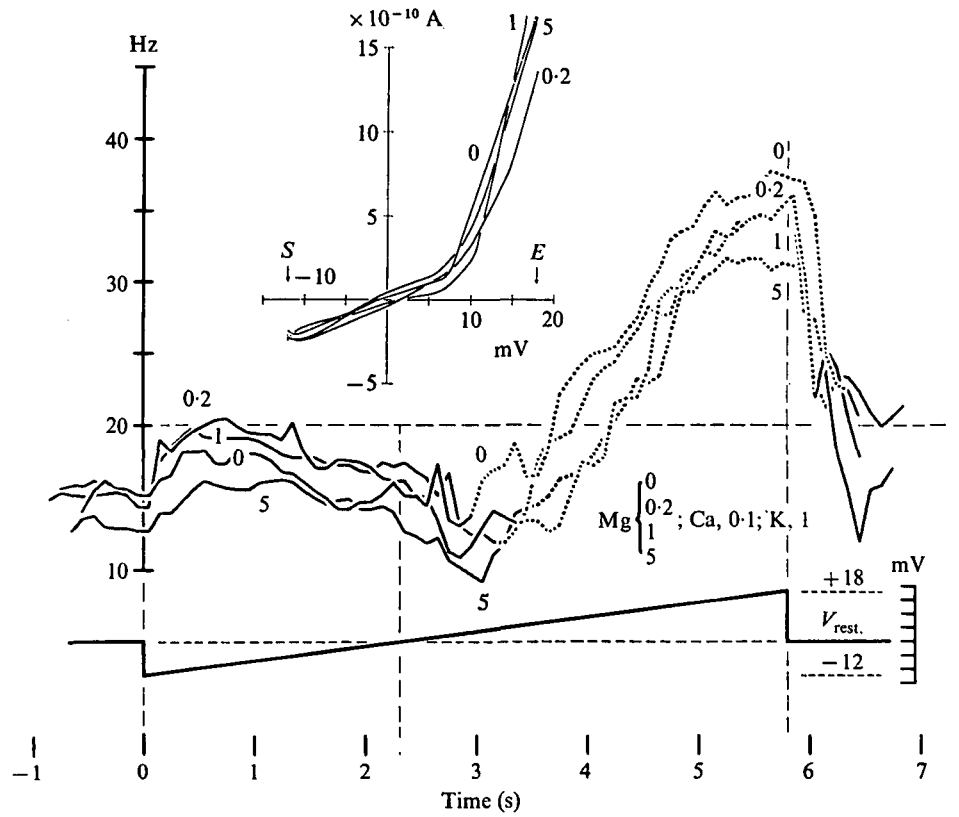

Fig. 5. Magnesium series with holding potential equal to resting potential in each solution. Each curve averaged from frequency readings in 5 cells, each of which was exposed to 4 solutions with magnesium at 0·0, 0·2, 1 and 5 mM plus the standard composition of 0·1 mM-Ca and 1 mM-K. Mean resting potentials (mV): 0·0 Mg, −63; 0·2 Mg, −58·7; 1 Mg, −44·8; 5 Mg, −31·8. Inset: corresponding averaged *I/V* curves.

whereas Mg did not. Prestimulus frequency was similar at similar concentrations of the two ions, differing by no more than 2 Hz (Table 2). Hyperpolarization demonstrated a clear difference in the effects of Ca and Mg upon ciliary activity (1 mM: 46% versus 11% increment; 5 mM: +23% versus −11%). Ca was more effective in shifting the frequency minimum to more positive potentials (1 mM: △4·9 mV versus △2·2 mV). Mg kept the frequency minimum low (reductions up to −31%), while elevation of Ca level raised the frequency minima (up to +58%; compare Table 2). The frequency of reversed beating induced by depolarization showed no significant alteration when Ca was replaced with Mg.

3. *Responses to voltage ramps with fixed holding potential*

Ciliary responses to ionic changes subsided while the cell equilibrated. Since this involved a change in resting potential, it is to be expected that membrane conductances were altered. Alterations of the potential of individual cells were prevented in the experiments described below by clamping the potential to a fixed value throughout each series of varied cation concentrations.

Table 4. *Modifications in* Paramecium *of electrical membrane parameters and ciliary responses to a voltage ramp by equilibration in solutions with varying* [*K*], *and* [*Ca*] *of* 1 *m*M

(Holding potential is equal to the resting potential in either 0·1 mM-Ca, 1 mM-K (series I) or 1 mM-Ca, 3·2 mM-K (series II).)

Series	Solution (mM) [K]	Holding potential (mV)	Resistance* at holding potential (megohm)	Voltage and current at minimal frequency (mV)	Voltage and current at minimal frequency (10^{-10} A)	Frequency (Hz) Pre-stimulus	Frequency (Hz) Maximum norm. beat	Frequency (Hz) Minimum	Frequency (Hz) Maximum rev. beat
I	0·44	−57·3	29	−43·5	−0·4	20	31	18	30
	0·95		30	−44·0	−0·4	19	32	16	27
	1·8		28	−46·2	−1·5	20	33	15	27
	3·2		29	−41·0	−0·6	21	35	14	20
II	0·44	−33·0	62	−30·3	+3·0	15	18	7	46
	0·95		120	−29·8	+1·6	14	17	2	44
	1·8		96	−28·2	+1·4	9	19	7	41
	3·2		48	−29·3	+0·2	10	17	10	39

* As determined at start of hyperpolarization.

3.1. *Change in* [*K*] *with constant* [*Ca*]

In two sets of experiments cells were equilibrated in two different solutions: one providing a high resting potential (K series I; 0·1 mM-Ca + 1 mM-K; average potential from 4 cells: −57·3 mV) and another giving a low resting potential (K series II; 1 mM-Ca + 3·2 mM-K; potential average from 2 cells: −33 mV). The holding potential in each series was set at the individual resting potentials in these solutions, so as to provide common physiological conditions, before the cells were exposed to the experimental solutions.

Electrical responses. In series I a constant low resistance was observed at all K concentrations (Table 4, compare with Table 1) combined with increased net inward current (inset Fig. 6, compare with Fig. 3). In series II the resistance was similar or increased with respect to the resting resistance in the equilibrating solution (compare Tables 3 and 1) and net outward current was increased at K < 3·2 mM (inset Fig. 7, compare with Fig. 3). As K concentration was decreased, I/V curves were shifted upwards on the current axis. A similar but smaller effect was seen in series I. In both series the rapid hyperpolarization from the holding level produced time-dependent inward current deflexions, the amplitudes of which decreased as potassium concentration was raised above or fell below 1·8 mM.

Ciliary responses. For cells exposed to series I, beating frequency prior to stimulation, and during hyperpolarization, was increased compared to cells in which the holding potential was set equal to the resting potential. During the voltage ramp maximal normal beating and minimal reversed beating occurred at the highest K concentration (3·2 mM; Tables 1, 4). In series II, the prestimulus frequency and hyperpolarization maxima were lower than normal, but depolarization maxima were higher than normal.

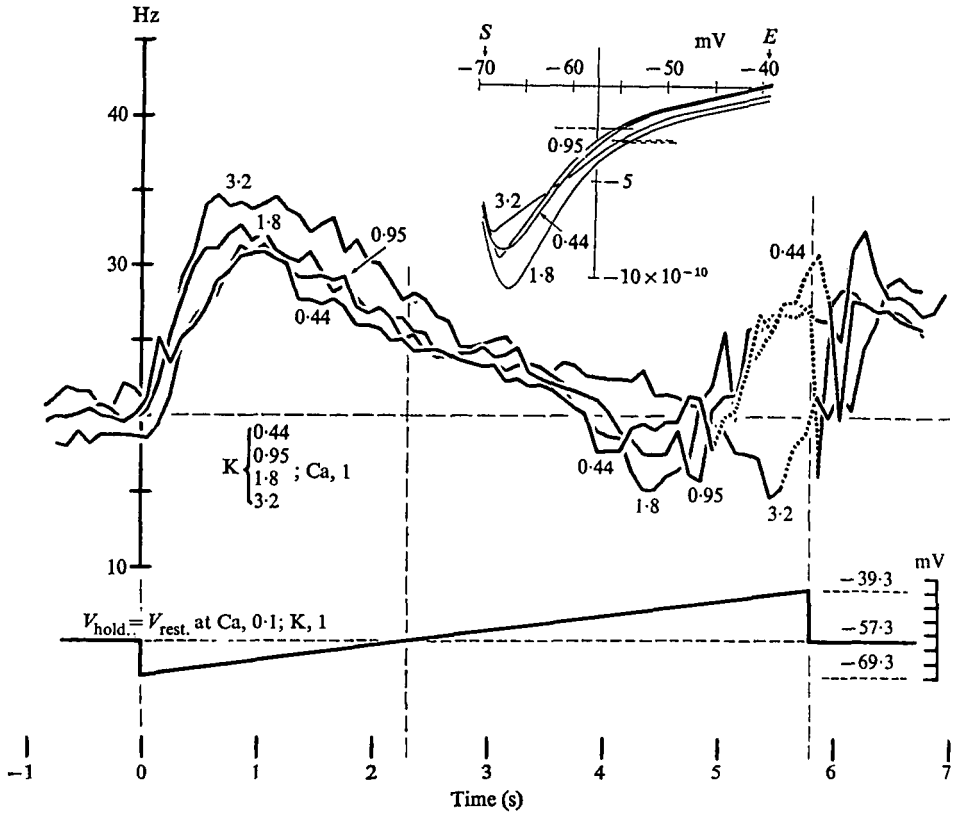

Fig. 6. Potassium series I with holding potential set throughout at the individual resting level in solution of 0·1 mM-Ca + 1 mM-K. Each curve averaged from 4 cells; each cell exposed to entire series. Mean resting potential: −57·3 mV. Inset: corresponding averaged *I/V* curves. Dashed horizontal lines intersecting curves show current level at holding potential.

3.2. *Combined effects on cilia of membrane potential and cation concentration*

Comparison of frequency/voltage curves generated in the same medium, but with holding potentials differing by 24 mV (Figs. 6, 7), shows that the 'V' of the frequency profile occurred at more positive potentials during the ramp, when the holding potential was more negative. The potentials at which the minimal frequencies occurred differed by 18 mV or less, which is smaller than the difference in the holding potentials (Table 4). Thus, the motor response of the cilia was not determined exclusively by the membrane voltage.

With both holding potentials, a sevenfold decrease in external K tended to reduce normal beating, and/or to increase reversed beating, of the cilia during the voltage ramp (Figs. 6, 7). No fixed correlation existed between the concentration and a certain ciliary response. However, an increase in concentration acted to partly offset effects of decreasing membrane negativity on the motor response. This effect of concentration is still more conspicuous with Ca and Mg (Figs. 8, 9).

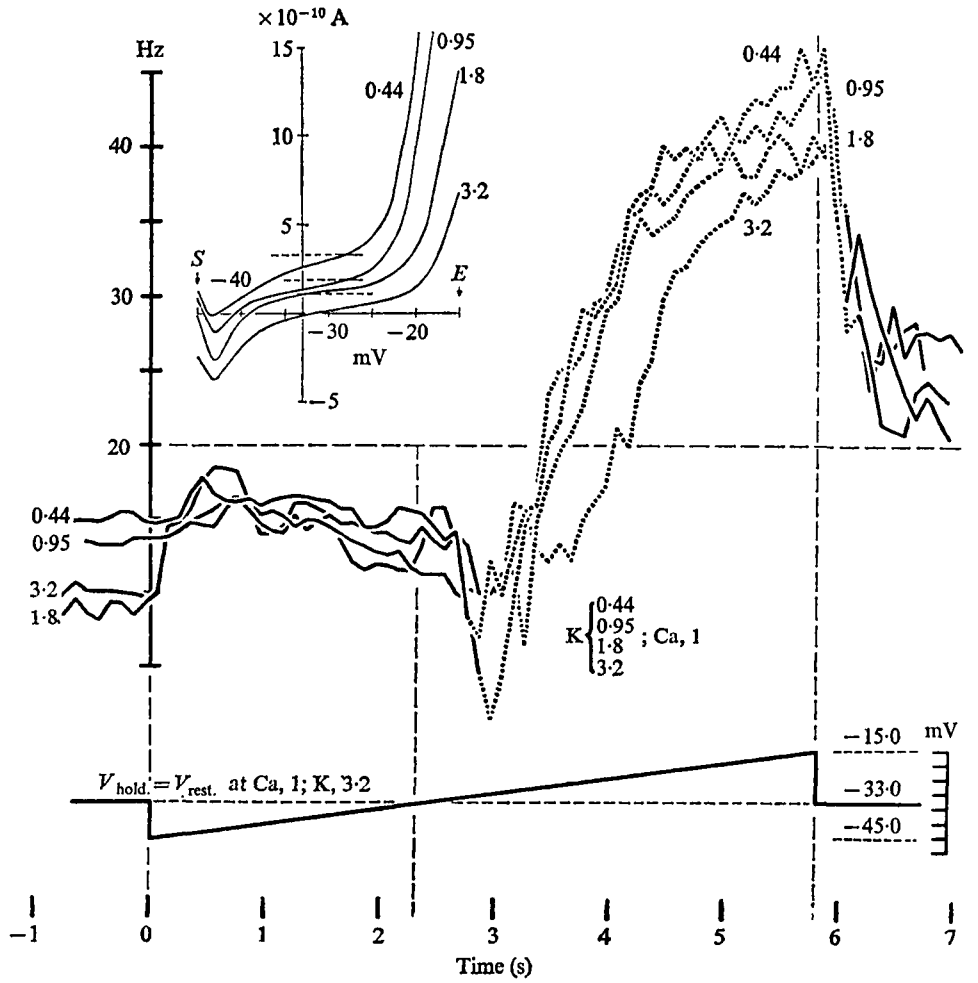

Fig. 7. Potassium series II with holding potential fixed throughout at individual resting level in 1 mM-Ca + 3·2 mM-K. Each curve averaged from 2 cells. Mean resting potential: −33 mV. Inset: corresponding averaged I/V curves (see Fig. 6 for details).

3.3. *Change in [Ca] with constant [K]*

Electrical responses. Cells were equilibrated and clamped at the individual resting potentials in 0·1 mM-Ca + 1 mM-K (average potential from 5 cells: −59 mV; Table 5). An increase in Ca concentration reduced the membrane's resistance and strongly increased net inward current (inset Fig. 8, compare Table 2). With rising Ca the 'sigmoid' I/V curve increasingly shifted toward more positive potentials, intersecting the zero current line approximately at resting potential levels.

Ciliary responses. Increase in Ca concentration above that in the equilibrating solution considerably raised the prestimulus beating frequency (Table 5, Fig. 8). Maximal frequency during hyperpolarization was produced in 1·1 mM-Ca. Reversed beating was observed only in 0·3 and 0·1 mM-Ca, and was maximal at the lowest concentration. Increase in Ca shifted the frequency minima towards less negative potentials and resulted in there being less frequency change during stimulation.

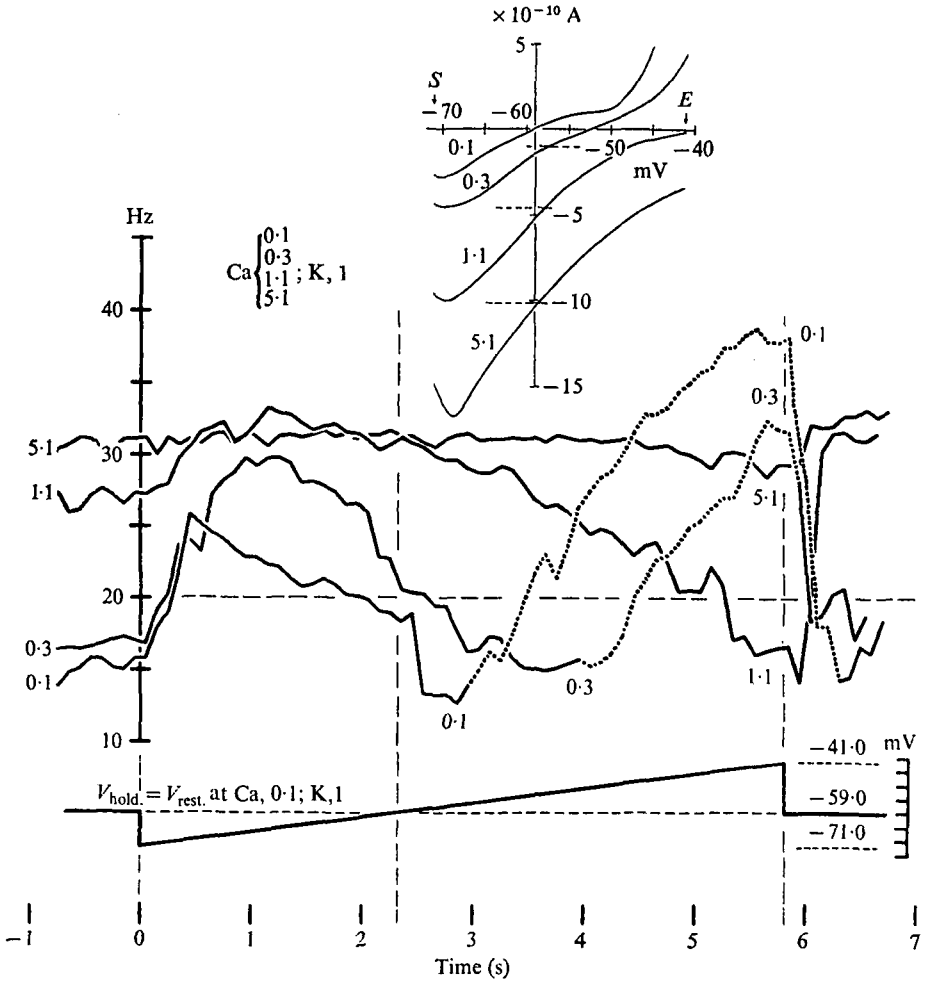

Fig. 8. Calcium series with holding potential equal to individual membrane resting level in solution of 0·1 mM-Ca + 1 mM-K. Each curve averaged from readings in 5 cells. Mean resting potential: −59 mV. Inset: corresponding averaged *I/V* curves (see Fig. 6 for details).

3.4. *Comparison between frequency/voltage curves with the same* $[K]/[Ca]^{\frac{1}{2}}$ *ratio*

Pairing of frequency curves from the K series I and the Ca series (Figs. 6, 8) with identical $[K]/[Ca]^{\frac{1}{2}}$ ratio revealed no similarity in the ciliary responses, nor did a decrease in the ratio generate common tendencies in the activity of the cilia. This reinforces the earlier conclusion (section 2·3) that there appears to be no consistent correlation between the $[K]/[Ca]^{\frac{1}{2}}$ ratio and electrically evoked motor responses of the cilia.

3.5. *Change in* [*Mg*] *with constant* [*K*] *and* [*Ca*]

Electrical responses. Equilibration and subsequent clamping to the individual resting potential was in solution of 0·1 mM-Ca and 1 mM-K (average potential from 4 cells: −60·3 mV). In all Mg solutions the resistance at the holding potential was

Table 5. *Modifications in* Paramecium *of electrical membrane parameters and ciliary responses to a voltage ramp by equilibration in various [Ca] and [Mg]*

(Holding potential equal to resting potential in 0·1 mM-Ca and 1 mM-K.)

Solution (mM)		Holding potential	Resistance* at holding potential		Voltage at minimal frequency	Frequency (Hz)				
[Ca]	+ [Mg]	(mV)	(megohm)	Δ (%)	(mV)	Pre-stimulus	Maximum norm. beat	Δ (%)	Minimum	Maximum rev. beat
0·1†	—	−59·0§	45	—	−56·3	16	26	—	13	38
0·1‡	—	−60·3§	60	—	−56·6	17	23	—	13	42
0·3	—	—	35	−22	−52·1	17	30	+15	15	32
0·1	0·2	—	37	−38	−53·5	13	27	+17	12	37
1·1	—	—	24	−47	?	27	33	+27	?	—
0·1	1·0	—	37	−38	?	22	33	+43	?	—
5·1	—	—	26	−42	?	31	32	+23	?	—
0·1	5·0	—	36	−40	?	32	33	+43	?	—

* As determined at start of hyperpolarization.
† Reference for Ca-series.
‡ Reference for Mg-series.
§ Equal to resting potential in 0·1 mM-Ca + 1 mM-K.

equally decreased with respect to the resting resistance (Table 5, compare with Table 2). Inward net current increased with an elevation of Mg concentration (inset Fig. 9), an increase similar to that found with Ca. Mg-dependent shifts in the *I/V* curves resemble those seen in the Ca series and suggest similar mechanisms. Hyperpolarization produced weak time-dependent inward deflexions of current which were absent in 5 mM-Mg (Fig. 9).

Ciliary responses. Prestimulus beating frequency rose at the higher Mg concentrations with respect to zero Mg (Fig. 9, Table 5). After onset of the potential ramp the frequency of normal beating rose to peak values which were maximal at 1 mM-Mg. The properties of the frequency response in different Mg concentrations were similar to those observed with varied Ca, i.e. increasing Mg made the slope of the frequency profile more shallow and shifted its minima toward more positive potentials.

3.6. *Comparison of Ca series with Mg series*

At similar holding potentials the partial replacement of Ca with the same amount of Mg did not consistently increase or decrease the membrane resistance (Table 5). The resistance was most strongly reduced at higher cationic concentrations. *I/V* characteristics were shifted toward positive potentials by Mg as well as by Ca (Figs. 8, 9). Greater currents in the Ca series are largely due to somewhat smaller input resistances of cells in this series (see reference pair in Table 5). The main differences between the sets of Ca and Mg curves occurred in the delayed current activation following hyperpolarization. Frequency profiles produced at the same divalent cation concentration, but with different concentrations of Ca and Mg, differed from each other only at low Ca (0·3 mM) and low Mg (0·2 mM). Thus, these

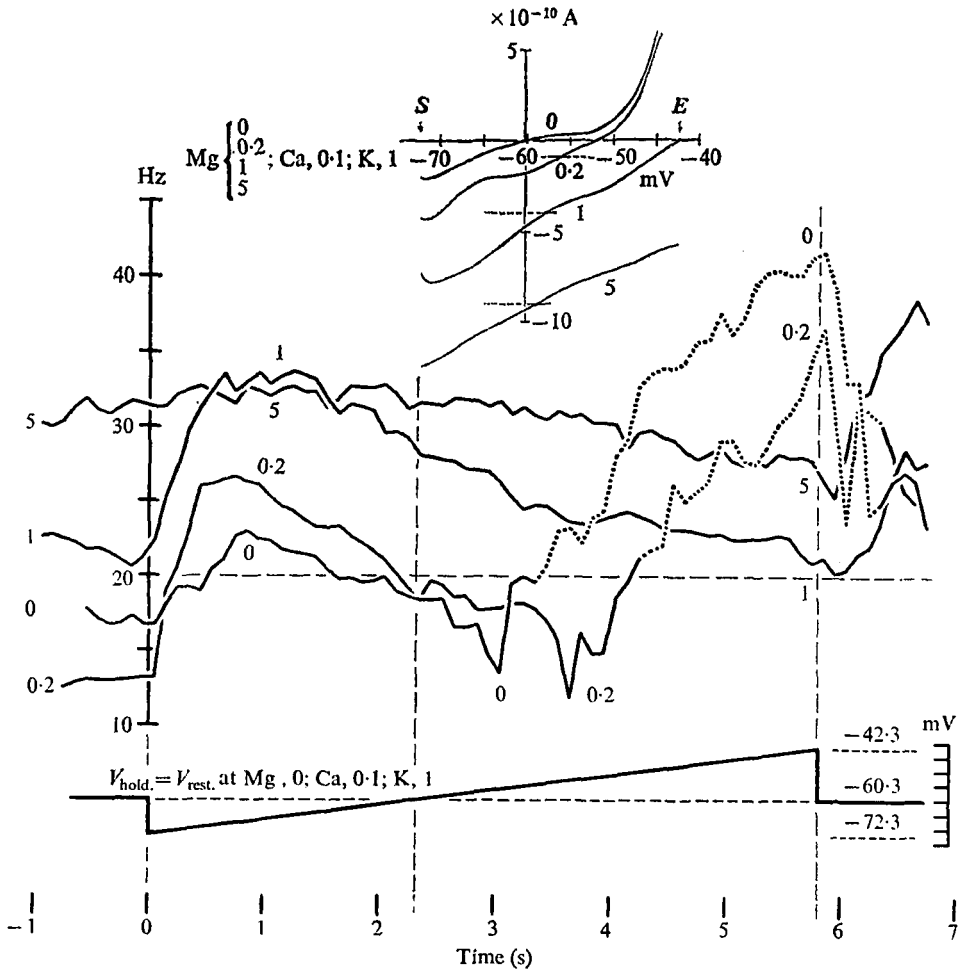

Fig. 9. Magnesium series with holding potential equal to the individual resting potential in the solution of 0·1 mM-Ca + 1 mM-K. Each curve averaged from readings in 4 cells. Mean resting potential: −60·3 mV. Inset: corresponding averaged *I/V* curves (see Fig. 6 for details).

specific effects of Ca on ciliary activity decreased with increasing displacement of the potential from the resting value.

DISCUSSION

A. *Effects of cations on electrical responses*

Resting potential. Increasing the concentration of K, Ca or Mg usually decreased the resting potential (V_{mr}), in accordance with findings by Naitoh & Eckert (1968). V_{mr} may become less negative as a result of decreased I_K, either through reduction in g_K by raised Ca and Mg concentrations (Frankenhaeuser & Hodgkin, 1957), or through reduction in E_K following an elevation in external K concentration. According to cation flux measurements in *Paramecium aurelia*, increasing K or Ca inhibits K efflux (Browning & Nelson, 1976*b*). A decrease in negativity of V_{mr} may also result

from increased g_{Ca}. A transient or continued depolarization of the membrane raises g_{Ca} which induces reversed beating of the cilia in *Paramecium* (Eckert, 1972; Naitoh, Eckert & Friedman, 1972; Machemer & Eckert, 1973, 1975). In the present study, however, no ciliary reversal occurred in conjunction with the positive shift in V_{mr} seen with raised external cationic strength. Presumably, net inward Ca current at diffe ent resting potentials is kept at low levels.

Membrane resistance. Raised concentrations of K, Ca or Mg increased the resting resistance, followed by a decrease, or *vice versa* (Tables 1, 2). Similar bimodal alterations of resistance have been shown in the unclamped *Paramecium* membrane (Naitoh & Eckert, 1968). In the present experiments, the resting resistance decreased with depolarization of the membrane (Table 1), suggesting potential-dependence of the resting conductance, predominantly g_K, similar to that in the squid axon (Hodgkin, 1958). The relatively small changes in resting resistance that were found in the Ca and Mg series (Table 2) might be explained by assuming that a potential-dependent increase in membrane conductance was offset, in part, by a Ca- and Mg-dependent change in conductance.

Steady-state membrane currents. An increase in inward current with hyperpolarization may resutl from an increase in net inward I_K or I_{Ca}. According to evidence in reactivated models of *Paramecium* (Naitoh & Kaneko, 1972) and in living cells (Machemer, 1974; Machemer & Eckert, 1975), beating of cilia in the normal direction concomitant with hyperpolarization indicates a reduced internal Ca concentration. Two alternative explanations are suggested for the inward current: (1) Ca which entered the cell across a leaky membrane, and/or through raised E_{Ca}, increases the rate of Ca pumping. Both I_K and I_{Ca} are main components of the inward current. (2) The calcium conductance is decreased. Inward current is predominantly carried by K.

The observation that an increase in external Ca augments inward current seen upon hyperpolarization (Figs. 4, 8) suggests that the currents may be carried by Ca. However, there is no concomitant reversal in the direction of beating of the cilia which would signal increase in $[Ca]_i$. On the contrary, *normal* beating frequency during hyperpolarization is raised with $[Ca]_o$, so that $[Ca]_i$ is presumably decreased. This may be because of the activity of some calcium pump such as has been described in nervous tissue (Baker, 1972). In squid axons, an increase in external calcium produced a decrease in the inward current associated with a hyperpolarizing pulse (Frankenhaeuser & Hodgkin, 1957).

The alternative explanation, conductance-limited decrease in $[Ca]_i$, is supported by the occurrence and magnitude of time-dependent inflexions of inward current after rapid hyperpolarization which are commonly ascribed to rectifying properties of the potassium channel. Rectification increased with raised $[Ca]_o$ (Figs. 4, 8), but not with modified $[Mg]_o$ at constant $[Ca]_o$ (Figs. 5, 9). Rectification decreased with elevated $[K]_o$, when hyperpolarization occurred from the membrane resting potential (Fig. 3). Calcium increases rectification in *Loligo* nerve (Steinbach, Spiegelman & Kawata, 1944). An increase in intracellular calcium has been found to raise the conductance to outward K fluxes in certain tissues (Whittam, 1968; Lew, 1969; Meech & Strumwasser, 1970; Grabowski, Lobsiger & Lüttgau, 1972; Junge, 1972; Krnjević & Lisiewicz, 1972; Jansen & Nicholls, 1973). A similar $[Ca]_i$-dependent component of the potassium conductance is suggested by the parallel slow time

courses of ciliary frequency and membrane currents in the step-clamped *Paramecium* (Machemer & Eckert, 1975). A possible explanation of the observed effect of $[Ca]_o$ on rectification is that Ca augments hyperpolarization-dependent reduction of $[Ca]_i$, which then reduces the outward component and/or increases the inward component of the potassium current.

B. *Dependence of ciliary responses on cations*

Effects with fixed holding potential. An increase in cation concentration raised the frequency of normal beating ($f_{n.b.}$) and/or decreased the frequency of reversed beating ($f_{r.b.}$) during the voltage ramp (Figs. 7–9). Evidence has been accumulated that reversed beating of the cilia in *Paramecium* results from increased internal Ca (Naitoh & Yasumasu, 1967; Naitoh, 1968; Eckert, 1972; Machemer & Eckert, 1973). Asymmetries in the ciliary motor response to depolarization and hyperpolarization (Machemer, 1974, 1975; Machemer & Eckert, 1975) and evidence from cell reactivation studies (Naitoh & Kaneko, 1972) show that increased frequency of normal beating is associated with decrease in $[Ca]_i$. Thus, steps between an increase in external cation concentration ($[C]_o$) and the ciliary response occur in the following sequence: $\uparrow [C]_o \rightarrow \downarrow [Ca]_i \rightarrow \uparrow f_{n.b.}$ or $\downarrow f_{r.b.}$. Similar frequency responses produced by equimolar Ca and Mg (Figs. 8, 9) suggest that these two ions have similar effects on $[Ca]_i$. However, Ca appears to more effectively promote reduction in $[Ca]_i$ than does Mg, if negative shifts from the resting potential are small (Figs. 4, 5).

Cation-stabilized ciliary response

The frequency response remained stable in different concentrations of K, Ca and Mg with a holding potential equal to the resting potential (Figs. 2–5). Data given in this paper, and recent evidence in *Paramecium* (Machemer, 1975; Machemer & Eckert, 1975), show that a clamped depolarization of the membrane reduces the frequency of normal beating or increases the frequency of reversed beating. It follows that depolarization has effects on ciliary activity which are opposite to those of an increase in external cation concentration. The converse will be found with hyperpolarization. Stabilization of the ciliary response with different cation concentrations may be due, in part, to a stabilized calcium conductance. Frankenhaeuser & Hodgkin (1957) observed in the squid axon that an increased concentration of Ca or Mg had an opposite effect on inward sodium current to the effect of depolarization. Recent direct measurements of the passive cation fluxes across the *Paramecium* membrane (Browning & Nelson, 1976*a*) showed that divalent cations decreased, and monovalent cations increased, the Ca influx at given concentrations. With an increase in $[K]_o$ as well as $[Ca]_o$, the influx of Ca at 0 °C increased or decreased depending on the concentrations of external K and Ca. These results suggest that external cations can decrease the internal Ca concentration via a decrease of the calcium conductance.

Some of the possible correlations between the concentrations of external K, Ca and Mg, the membrane potential and the ciliary frequency and direction of beating in *Paramecium* are illustrated in Fig. 10. The diagram explains how an increase in these external cations can reduce the resting potential but not essentially modify the ciliary activity of the resting cell. Contributions of other factors in the interaction of cations and membrane potential, such as the determination of I_K by the potential

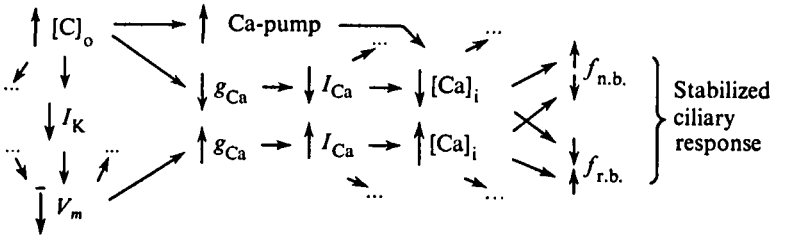

Fig. 10. Proposed main steps in the interaction of external cations ($[C]_o$) and membrane potential in stabilizing the frequency of normal beating ($f_{n.b.}$) or of reversed beating of the cilia ($f_{r.b.}$). The flow diagram neglects effects along various pathways (→...) which are considered to be less important in regulating the cilia. I_K, outward component of K fluxes. I_{Ca}, inward component of Ca fluxes.

and conductance, and the effects of I_{Ca} on V_m, are neglected for simplicity. Allowing for such inaccuracies the diagram predicts shifts in frequency with changed external cations at fixed V_m (Figs. 8, 9), and with changes in V_m in constant external solutions (Figs. 2–9).

C. *Is the* $[K]/[Ca]^{\frac{1}{2}}$ *ratio relevant to ciliary activity?*

Explanations of the control of cilia according to an ion-exchange hypothesis (Jahn, 1962; Naitoh, 1968) have been concerned with the duration of reversed swimming in *Paramecium*, which indicates the start and end of a gradually modified ciliary reversal response (Machemer, 1974). The present data allow comparison of frequency and directional responses in electrically stimulated cells which were equilibrated with the ratio $[K]/[Ca]^{\frac{1}{2}}$ held constant. If the amount of bound Ca in a proposed cation-exchange system has direct relevance to ciliary activity, then stimulation of membranes, immersed in solutions with the same $[K]/[Ca]^{\frac{1}{2}}$ ratio, should produce similar responses of the cilia. However, stimulation at different K and Ca concentrations, at the same holding potential and with identical voltage ramps, elicited different ciliary responses. The cation-exchange hypothesis of ciliary stimulation, therefore, appears not to be applicable to ciliary motor responses evoked by slow voltage ramps. The empirical relevance of the $[K]/[Ca]^{\frac{1}{2}}$ ratio to ciliary activity may be produced, in part, through the different effects of Ca and K on the calcium conductance.

D. *Roles of internal Ca and Mg in ciliary activity*

In the present experiments changes in external Mg concentrations did not exhibit specific effects on ciliary activity. Two possible explanations are: (1) internal Mg plays no role in ciliary movements; (2) internal Mg does not undergo significant change in concentration during bioelectric activity. The first assumption appears to be ruled out since ionic Mg is a co-factor in ATP-reactivation of ciliary systems (Brokaw, 1961; Gibbons, 1965; Eckert & Murakami, 1972; Gibbons & Gibbons, 1972) and, in Triton-extracted *Paramecium*, the restitution of ciliary activity requires ATP and Mg in concentrations of a few millimolar (Naitoh & Kaneko, 1972, 1973). The latter findings support explanation (2) since external Mg concentrations of up to 5 mM, as pointed out by Eckert & Machemer (1975), may be insufficient to raise the internal Mg activity. Naitoh & Kaneko found that Ca could not replace

Mg in the reactivation of the cyclic motor response, but induced a reversal of the beat orientation at concentrations > 10^{-6} M. Ca,on the other hand, modifies frequency in reactivated ciliary systems. In Triton-X extracted ciliated epithelium of *Necturus*, at constant Mg and ATP concentrations, peak frequency occurs with Ca slightly below 10^{-6} M. (Eckert & Murakami, 1972). A similar, but more pronounced, Ca-dependence of reactivated cilia occurs in *Paramecium*: frequency having been shown to be maximal at 10^{-6} M-Ca, dropping toward a minimum at close to 10^{-7} M, and rising again with further decrease in Ca concentration (Naitoh & Kaneko, 1972). This minimum in frequency, with the Ca level raised slightly above that which produces normal swimming (10^{-8}–10^{-7} M; Naitoh & Kaneko, 1972), is reminiscent of the ciliary inactivation observed in live *Paramecium* with small depolarizations of the clamped membrane (Machemer, 1975; Machemer & Eckert, 1975; Fig. 2 this paper), and the inactivation during slow recovery of beating in the normal direction following a reversal induced by depolarization (Machemer, 1974). On the basis of available evidence it is proposed, therefore, that membrane-controlled inward Ca fluxes and internal steady Mg levels produce varying ratios of the intracellular activities of Ca and Mg, and that these ratios determine the frequency and orientation of the cilia. According to this view, beating of cilia at high frequency in the normal direction is primarily Mg activated, while high frequency of beating in the reversed direction is Ca-Mg activated. The observed inactivation of the cilia during weak depolarization may result from inhibition of the Mg system with still insufficient activation of the Ca system.

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