J. exp. Biol. (1982), 97, 311–324
With 10 figures
Printed in Great Britain

OSMOTIC TOLERANCE OF Ca-DEPENDENT EXCITABILITY IN THE MARINE CILIATE *PARAMECIUM CALKINSI*

BY JOACHIM W. DEITMER AND HANS MACHEMER

Abteilung Biologie, Ruhr-Universität, D-4630 Bochum, Federal Republic of Germany

(*Received 12 June 1981*)

SUMMARY

The electrical membrane properties of the marine (brackish-water) ciliate *Paramecium calkinsi* were investigated under constant-current and voltage-clamp conditions, using two intracellular microelectrodes. The action potential and membrane currents were extremely tolerant to changes in the salinity of the bathing medium. Current–voltage relationships exhibited a moderate inward-going rectification of the membrane upon hyperpolarization, and a prominent outward-going rectification upon depolarization of the membrane. Ion substitution experiments showed that the electrically excitable response is a graded Ca^{2+}-action potential, similar to that found in freshwater ciliates.

INTRODUCTION

Electrically excitable cells may respond to changes in the osmotic or ionic concentration of their environment either with rapid adaptation or with loss of their excitability (which may involve irreversible damage). In unicellular organisms, such as ciliate protozoans, ionic and osmotic regulatory mechanisms are limited exclusively to the cell membrane. Ciliates usually tend to maintain a hyperosmotic cytoplasm as compared to the external medium (see, for example, Prusch, 1977). Electrophysiological work on *freshwater* ciliates has demonstrated that these cells, like other 'euryhaline' freshwater animals, can tolerate relatively large variations in concentration and composition of the external solution without harm to their electrical excitability (Naitoh & Eckert, 1968; de Peyer & Machemer, 1977). *Marine* ciliates, which are abundant in the coastal and brackish-water regions, have not yet been investigated electrophysiologically. These cells may encounter a wide salinity range in the sea water, similar to metazoan animals in the same habitat.

The present paper describes a first electrophysiological investigation on a marine ciliate under conditions of varying ionic and osmotic environments.

METHODS

Cells. Paramecium calkinsi was reared in a culture solution consisting of 8 or 20 g/l Tropicmarin (corresponding to approximately 20% and 50% artificial sea water (ASW) as given below), 2 mM-Na_2HPO_4 buffer (pH 7·6–7·8) and 1·25 g/l Cerophyl.

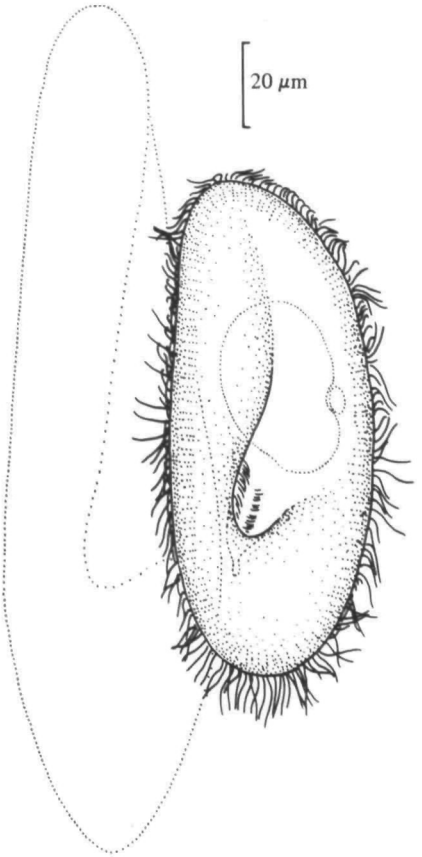

Fig. 1. *Paramecium calkinsi* (ventral view, anterior end up). The cell is slightly depressed in the dorso-ventral axis and smaller as compared to the freshwater species *P. caudatum* (see dotted outline for comparison). The total surface area is $3{\cdot}5 \times 10^{-4}$ cm^2 and thus approximately 50% that of *P. caudatum* (Machemer & Ogura, 1979). A typical oral groove leads from the anterior end toward the recessed cell mouth. Cilia of 10 μm length are equally spaced on the surface membrane and metachronally co-ordinated during activity. The most posterior cilia are immobile. Drawing after *in vivo* photographs taken by R. H. F. Ruhnke.

To adapt the cells to different salinities (equivalent to 25%, 75% and 100% ASW), the 20 g/l Tropicmarin solution was gradually diluted with distilled water, or concentrated with a 40 g/l Tropicmarin solution, at 24–48 h intervals, to give cultures in 10 g/l and 30 g/l Tropicmarin solution (corresponding to 25% and 75% ASW, respectively) after 1 week, and cultures in 40 g/l Tropicmarin solution (corresponding to 100% ASW) after 2 weeks. For experimentation cells were selected which had been equilibrated for at least 48 h in 10, 20, 30 or 40 g/l Tropicmarin solution.

Electrical recording. Single specimens of *Paramecium calkinsi* (between 110 and 140 μm in length, Fig. 1) were impaled with two single glass microelectrodes. One microelectrode was filled with 2 M-K-citrate (50–70 MΩ) and used for current injection, the other microelectrode was filled with 3 M-KCl (40–80 MΩ) for voltage recording. A third microelectrode, filled with 3 M-KCl, was positioned just outsi

the cell. The membrane potential was measured as the voltage difference recorded by the intracellular and the extracellular KCl-microelectrodes.

The design for the injection of constant current and membrane voltage recording was essentially the same as described by Naitoh & Eckert (1972). For membrane voltage-clamp a high-gain differential amplifier (AD 171 K) was used. The input resting resistance was determined by injecting a small hyperpolarizing constant-current pulse (0·2 nA) of 80–150 ms duration. In the voltage–clamp experiments the membrane was held at its resting potential ('holding potential'). The voltage was changed stepwise for approximately 60 ms by + 5 mV up to + 30 mV and to − 40 mV. Membrane currents were monitored by a current–voltage converter connected to the bath. The maximum ionic currents, i.e. the peak early inward current and the steady-state current, were thus measured. For current–voltage relationships the peak early inward current was corrected for leakage current (about 0·5 nA or less for a ± 10 mV voltage step).

Solutions. For the experiments, the 100% ASW had the following ionic composition (in mM): NaCl, 434; KCl, 10; $CaCl_2$, 10; $MgCl_2$, 53; $NaHCO_3$, 2·5; Hepes buffer (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid), 2; the pH being 8·0–8·1. The various dilutions of the ASW (20%–75%) were prepared from this 100% ASW by adding the appropriate amounts of distilled water. The Na^+-free ASW contained choline-Cl for NaCl; in the Ca^{2+}-free ASW $CaCl_2$ was exchanged for the osmotically equivalent amount of NaCl. In one test solution the Ca^{2+} concentration was increased by replacing $MgCl_2$ with $CaCl_2$ (thus having 32 mM-$CaCl_2$ and zero $MgCl_2$ in 50% ASW). In another test solution all $CaCl_2$ was exchanged for $SrCl_2$. If solutions were exchanged within the same experiment, the experimental chamber (volume less than 1·5 ml) was slowly perfused, exchanging at least 10 times the volume of the chamber. All experiments were performed with the experimental chamber held at 17–18 °C.

RESULTS

Electrical properties after long-term adaptation to different salinities

Specimens of *P. calkinsi* cultured in 50% ASW were slowly adapted to 25%, 75% and 100% ASW, as described in *Methods*. After equilibration the cells had a normal shape and size with no apparent signs of osmotic stress. The membrane resting potential was − 8 mV in 75% ASW and was slightly increased with rising salinity (− 10 mV in 100% ASW) or dilution (− 15·6 mV in 25% ASW; see Table 1). The resting input resistance was lowest in 100% ASW (11·7 MΩ) and rose to 23·2 MΩ in a salinity of 25% ASW (Table 1).

Injections of square, inward going, currents passively hyperpolarized the membrane, revealing membrane time constants between 10 and 15 ms (Fig. 2). Membrane depolarizations following small outward going currents were electrotonic and largely symmetric to hyperpolarizations up to positive shifts of 4–6 mV. With larger depolarizations of the membrane regenerative responses, 'graded action potentials', were elicited. The amplitude of these action potentials had peak amplitudes up to 30 mV with overshoots up to 20 mV depending on the resting potential and the stimulus current strength. During sustained depolarization the action potential was followed

Table 1. *Resting potential and input resistance in* Paramecium calkinsi *in different concentrations of artificial sea water (ASW)*

(Values of the input resistance were derived from the shift in voltage following 80–150 ms hyperpolarizing currents of 0·2 nA.)

	Cation concentration (mM)							
ASW (%)	Na^+	K^+	Ca^{2+}	Mg^{2+}	V_m (mV)	n	R_{inp} (MΩ)	n
25	109	2·5	2·5	13·3	−15·6±1·3	10	23·2±4·7	10
50	218	5·0	5·0	26·5	−12·2±2·1	18	16·6±4·7	16
75	327	7·5	7·5	39·8	−8·0±1·0	3	13·3±5·8	3
100	436	10·0	10·0	53·0	−10·0±1·0	3	11·7±2·9	3

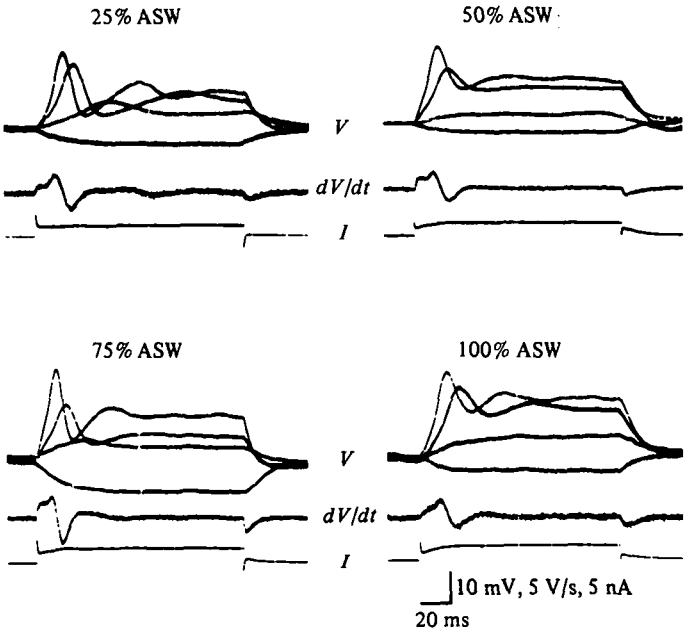

Fig. 2. Membrane potential changes of *Paramecium calkinsi* recorded in artificial sea water (ASW) of different salinities. The superimposed depolarizing and hyperpolarizing responses (upper traces) were evoked by constant current pulses. Graded action potentials were generated above a threshold depolarization of 4–7 mV. The lower trace shows the current pulse with the strongest amplitude given; the inflexion in the time derivative of the depolarization evoked by this current pulse (middle trace) illustrates the occurrence of the regenerative response. Note the similarity of the membrane responses in these very different ASW solutions.

by potential oscillations of decreasing amplitude before a steady-state level was achieved after 100–150 ms. The membrane excitability remained largely unaffected in external media containing between 100% and 25% ASW, which corresponds to a range in osmolarity between 1084 and 271 m-osmol (Fig. 2).

Recordings of membrane currents under voltage-clamp conditions at different salinities of the ASW (Fig. 3) agreed with the observations made in the unclamped

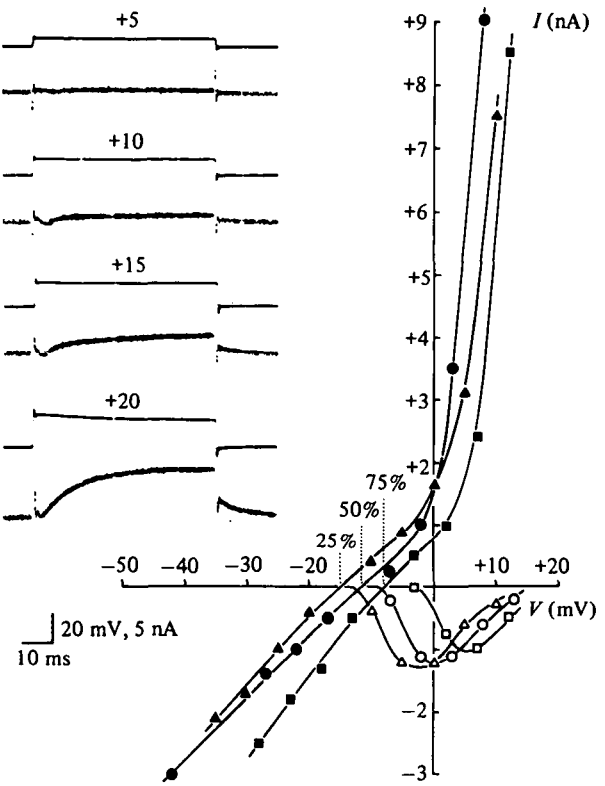

Fig. 3. Recordings of membrane currents (lower traces in column) during voltage steps (upper traces) from a holding potential equal to the resting potential in 50% ASW. The steady-state I–V relationship (filled symbols) shows pronounced outward rectification. Maximal early inward currents (open symbols) were recorded with depolarizations of 10–15 mV. Note the shift along the V axis of the early and the late I–V relationship in the different ASW dilutions (▲, △, 25%; ●, ○, 50%; ■, □, 75%).

membrane. The course of the I–V relationship remained essentially unchanged in the different salinities, 25%, 50% and 75% ASW. The relationships were shifted to more positive potentials as the ASW concentration was increased, as a result of the more positive holding (resting) potential in 50% and 75% ASW. The steady-state I–V relationships were almost linear in the hyperpolarizing direction (up to −30 mV) and exhibited a pronounced delayed rectification beyond 10 mV depolarization.

The early inward currents corrected for ohmic leakage were maximal with voltage steps of 10–15 mV, i.e. at membrane potentials between −5 mV and +5 mV.

Short-term osmotic tolerance of excitability

Paramecium calkinsi can rapidly adapt to moderate changes in salinity of the environment imposed within minutes on to the cell. Fig. 4 shows a series of typical membrane responses of one cell exposed to diluted ASW and subsequently to a raised concentration of the ASW (25% → 20% → 25% → 30%). After exchanging

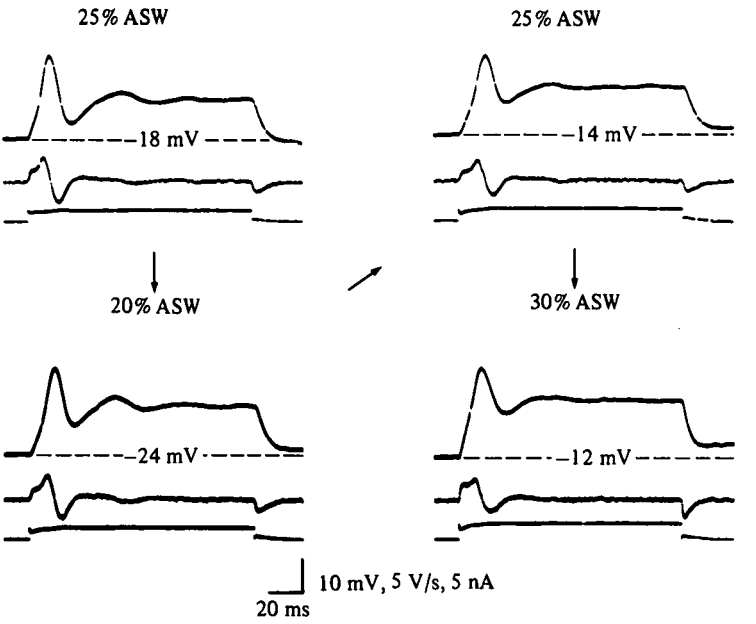

Fig. 4. Short-term osmotic tolerance of excitability. Records from one cell which had been equilibrated in 25 % ASW. The solutions were changed in sequence to 20 %, 25 %, 30 % and back to 25 % ASW at intervals of 3–5 min. Note the similarity of the action potentials arising from different resting potentials. After exposure to 30 % ASW the resting potential in 25 % ASW was −16 mV (traces not shown). The traces shown are voltage, dV/dt and current as in Fig. 2.

the bathing solution over a period of 2–3 min the membrane potential attained a new resting value in the next 3–5 min. Action potentials were evoked by depolarizing current pulses after stabilization of the resting potential. It is seen in Fig. 4 that the action potentials in 20% and 30% ASW were little, if at all, different from those in 25% ASW. The shifts in resting potential and in input resistance were largely reversible, although, after exchange lasting only 3–5 min, the range of fluctuation in potential was larger than in long-term adapted cells. In the example given in Fig. 4, the resting potential in 25% ASW was −18 mV in the fully equilibrated cell, but −14 mV after 20% ASW exposure and −16 mV after re-adaptation from 30% ASW (latter trace not shown). There was, however, a consistent tendency, like in long-term adapted cells, that both resting input resistance and resting membrane potential increased in lower salinity (20% ASW) and decreased in higher salinity (30% ASW).

Effects of Ca-removal

Replacement of the total Ca^{2+} with isosmotic amounts of Na^{+} (3·8 mM-NaCl for 2·5 mM-$CaCl_2$ in 25% ASW) suppressed the regenerative responses of the membrane (Fig. 5). The graded action potentials and oscillations of the membrane disappeared when the exchange of the solutions had been completed (approximately after 1 min, lower left traces of Fig. 5); at this time, membrane rectification was, however, sti

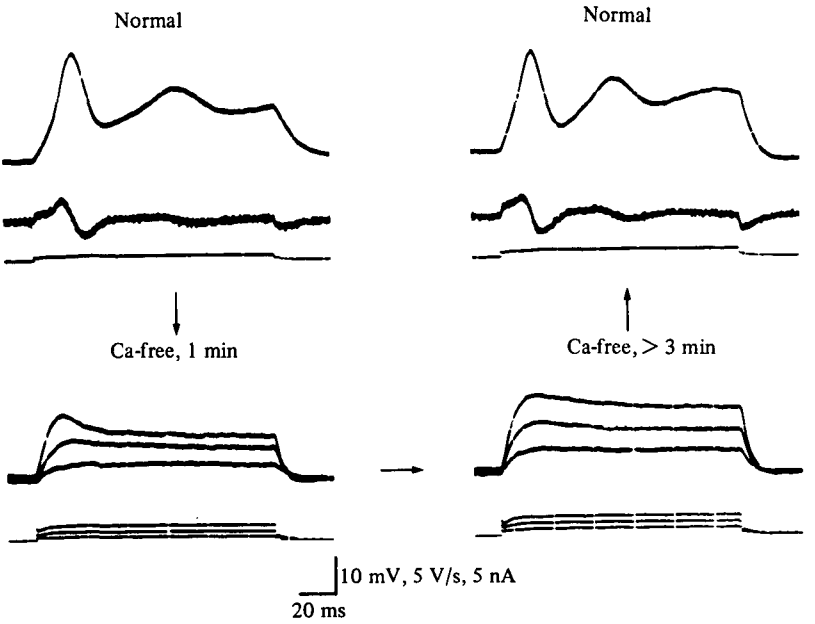

Fig. 5. Effects of Ca^{2+}-removal on the electrical excitability: Disappearance of the regenerative membrane response to depolarizing currents within 1 min (lower left trace) and reduction in delayed rectification with prolonged exposure to the Ca^{2+}-free 25 % ASW (Ca^{2+} replaced by Na^{+}). Normal excitability was re-established after re-addition of Ca^{2+} in the 25 % ASW control solution. All records are from one cell. Designation of traces as in Fig. 2.

perceptible. With continued exposure to the Ca-free solution (lower right traces of Fig. 5) the input resistance strongly decreased to approximately 30 % of the control value, and rectification almost disappeared, so that the I–V relationship was linearized (see Fig. 7B). The resting potential was slightly less negative (2–4 mV) than in the normal solution. No signs of membrane excitability were detectable even with depolarizations as large as 25 mV. The generation of action potentials was fully reversible after re-introduction of the normal Ca-containing ASW (Fig. 5, upper right traces). Membrane resting potential and input resistance also returned to their control values (– 16 mV, 20 MΩ). The experiments suggest that Ca^{2+} ions are necessary for the production of action potentials, and that Mg^{2+} ions are not involved.

When Sr^{2+} was substituted for Ca^{2+}, the electrical excitability of the membrane was essentially unmodified (Fig. 6). The membrane potential tended to be slightly more negative in the Sr^{2+}-containing ASW as compared with the normal, Ca^{2+}-containing ASW. Voltage-clamp experiments showed that the course of the steady-state I–V relationship, including the resting input resistance, exhibited prominent delayed rectification and insignificant inward rectification, and thus resembled that obtained in normal Ca^{2+}-containing 50 % ASW. We conclude that Sr^{2+} can substitute for Ca^{2+} as a charge carrier across the membrane of *Paramecium calkinsi.*

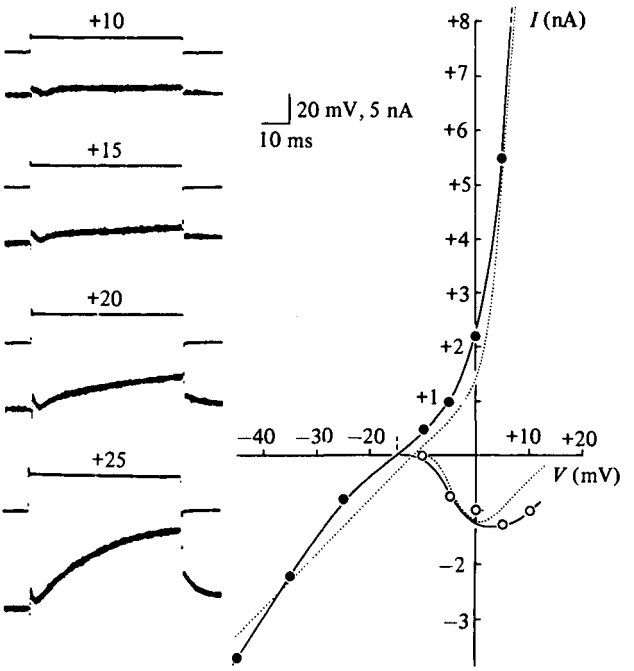

Fig. 6. Membrane currents in 50% ASW with Sr^{2+} substituted for Ca^{2+}. The column of traces shows current responses to depolarizing voltage steps between 10 and 25 mV. The courses of the early (open circles) and steady-state I–V relationship (filled circles) were essentially similar to those obtained in the normal Ca^{2+}-containing 50% ASW (dotted curves).

Effects of Na^+-removal

To test whether Na^+ could act as a charge carrier, external Na was replaced by choline (in 25% ASW). The membrane resting potential was up to 15 mV more negative in the Na^+-free ASW (−25 to −32 mV), and the input resistance rose threefold to approximately 70 MΩ. The depolarizing responses of the membrane following constant current stimulation were unaltered apart from an increase in relative threshold potential of up to 5 mV (Fig. 7A). The I–V relationship, as obtained from steady-state potentials during constant current injection, revealed a strong increase in rectification, in the inward as well as in the outward direction in the Na^+-free solution (Fig. 7B). This property, together with the raised input resistance, sharply contrasts to the I–V relationship from cells in Ca-free ASW, where the relationship was almost linear, and the input resistance was decreased with respect to that in the normal solution. The data suggest that Na^+ ions contribute to the maintenance of the resting potential of this membrane but are not involved in excitation.

Effects of raised external Ca^{2+}

The Ca^{2+} concentration of 50% ASW was raised more than sixfold (from 5 to 32 mM) by substitution of $CaCl_2$ for $MgCl_2$. Under these conditions the membrane hyperpolarized by 2–5 mV and the input resistance tended to increase slightly. T

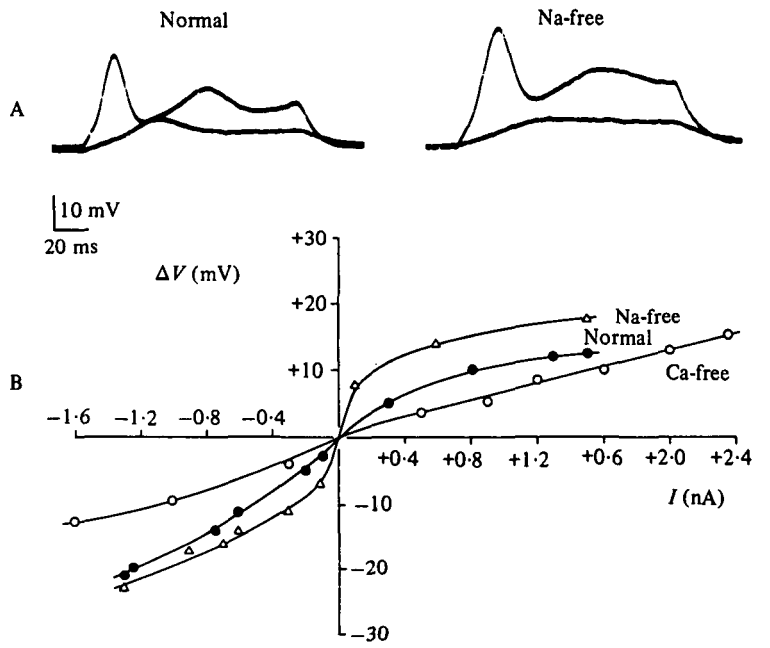

Fig. 7. Effects on electrical properties of Na^+-removal in 25% ASW. (A) Action potentials following outward current pulses in Na^+-free 25% ASW (Na^+ exchanged for choline) resembled those in normal ASW. (B) Typical steady-state I–V relationships obtained in normal, Na^+-free and Ca^{2+}-free ASW from constant current injection (130 ms). Note the increase in input resting resistance and rectification in the Na^+-free solution, and the decrease in input resistance and the linearization of the I–V relationship in the Ca^{2+}-free ASW.

regenerative membrane responses were enhanced in the Ca^{2+}-rich solution. The peak early inward current following positive voltage-clamp steps increased (Fig. 8) and approximately doubled as compared to that observed in the normal 50% ASW. The peak inward current was observed with 20 mV depolarizing voltage steps as compared to 10–15 mV steps for the peak inward current in the normal solution. Thus, while the steady-state I–V relationship was negatively shifted along the voltage axis in solutions of raised Ca^{2+}, the peak early inward current showed a positive shift along the axis.

Characteristics of the action potentials

For these experiments, 50% ASW with high Ca^{2+} contents (32 mM) was used to increase the amplitude of the action potential (see above). Action potentials elicited by 10 ms outward going current pulses reached peak amplitudes of up to 40 mV (Fig. 9A, B). The maximum rate of rise of the action potentials was up to 10 V/s, and the maximum rate of repolarization amounted to 12–15 V/s (see middle traces in Fig. 9A, B). A small negative afterpotential was followed by a prominent and long-lasting positive afterpotential. The amplitude and the duration of this positive late response were related to the size of the preceding action potential; they typically amounted to approximately 5 mV and 150 ms, respectively.

Graded action potentials including the afterpotential were also elicited at the end of

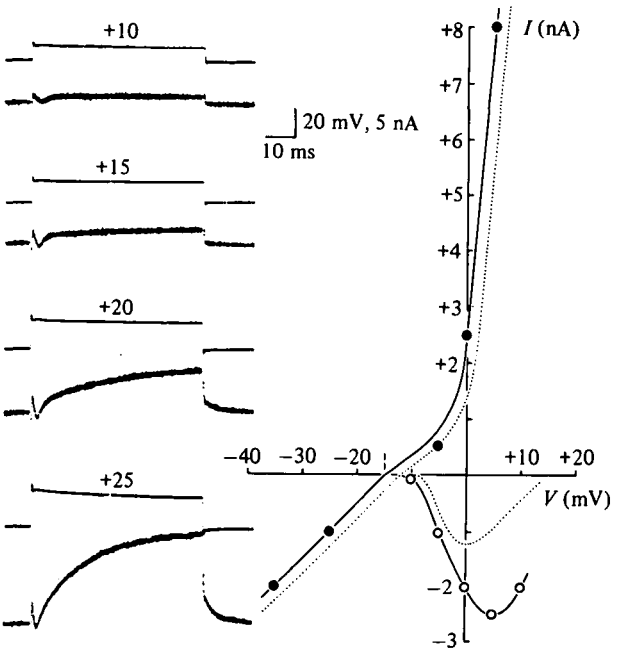

Fig. 8. Effects of raised Ca^{2+} concentrations on membrane currents. Early and steady-state *I–V* relationship from voltage clamp in solutions of 50 % ASW with Mg^{2+} replaced by Ca^{2+} (= 32 mM). Observe negative shift of steady-state *I–V* curve (filled circles) and the pronounced increase in the early-current peak (open circles) with respect to the *I–V* relationship in normal 50 % ASW (5 mM-Ca^{2+}, 26·5 mM-Mg^{2+}; dotted lines). Traces at left are sample recordings of membrane currents to depolarizing voltage steps in the high Ca^{2+} solution.

large membrane hyperpolarizations ($\geqslant$ 50 mV) produced by 100 ms inward current pulses ('anodal-break excitation', see Fig. 9C). The amplitude and rate of rise of these action potentials tended to increase with the amplitude of the preceding hyperpolarization. Large 'anodal-break excitations' were also followed by a pronounced positive afterpotential.

When identical depolarizing current pulses were given at intervals of less than 300 ms, the amplitude and the rates of rise and fall of the second action potential were reduced (Fig. 10A). This relative refractoriness of the membrane was most conspicuous at stimulus intervals of less than 150 ms. Small active responses following the second current pulse were detectable with stimulus intervals down to 30 ms (B–D). When the second stimulus occurred at intervals of less than 30 ms, the membrane response was only passive, i.e. no active response was elicited (E). These observations suggest that the period of relative refractoriness following an action potential is approximately 30–300 ms. The absolute refractoriness of the membrane falls within 30 ms after an excitation.

DISCUSSION

The main results of the present paper, for the membrane of *P. calkinsi*, show that (1) the electrical excitability, represented by graded action potentials, is virtuall

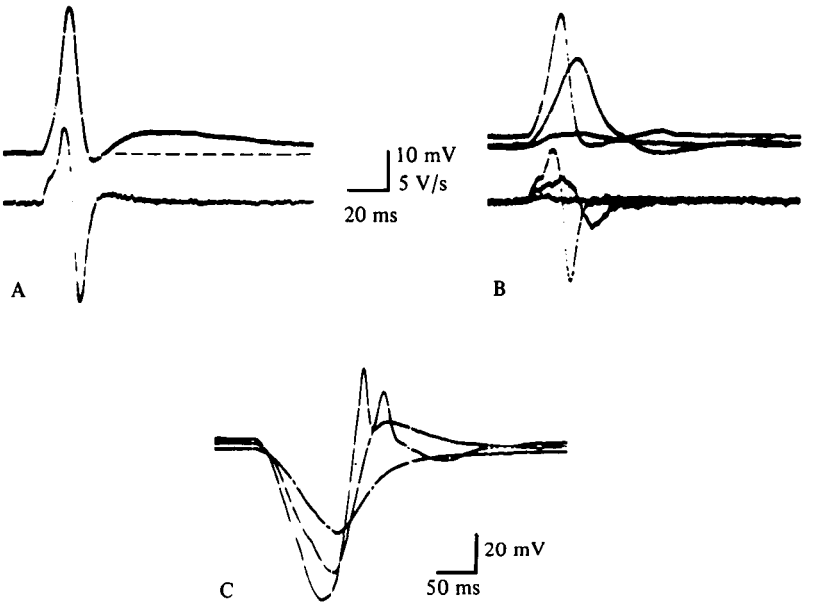

Fig. 9. Action potentials in high-Ca^{2+} solutions. Brief depolarizing stimulus currents (10 ms) led to action potentials of maximally 35–40 mV (A) which decreased in amplitude and rate of rise with decreasing stimulus intensity (B). After 100 ms hyperpolarizing current pulses of sufficient amplitude graded action potentials were elicited as a result of anode-break excitation (C). Note the negative and positive afterpotentials following all regenerative depolarizations.

unchanged in cells adapted to very different salinities and osmolarities of their surrounding medium, and (2) these action potentials are due to an inward membrane current carried by Ca^{2+} ions.

Osmotic tolerance of electrical excitability

The tolerance of the electrical excitability to a wide range of dilutions of the ASW bears some interesting similarities and differences compared to that of other invertebrate excitable cells. Metazoan invertebrates may regulate their extracellular microenvironment, e.g. by means of a blood-brain barrier, and thus protect the nervous system from large changes in ionic concentration and osmotic stress (see Abbott & Treherne, 1977; Treherne & Schofield, 1979). In some euryhaline osmoconformers, such as the polychaete annelids *Mercierella* and *Sabella*, progressive dilution of the blood ionic concentration is compensated by active extrusion of ions from the cells and by membrane hyperpolarization, in order to maintain the electrochemical ionic gradients used for electrical excitation (see e.g. Carlson & Treherne, 1977; Treherne & Pichon, 1978). In the stenohaline osmoconformer *Maia* (Crustacea) the spike-generating mechanism suffers irreversible damage by relative modest hyposmolarity (Pichon & Treherne, 1976). The marine euryhaline ciliate *Miamiensis* was found to maintain its cytoplasm hypertonic to the environment at all salinities (20%–100% ASW) by expelling water by means of contractile vacuoles (Kaneshiro, Dunham & Holz, 1969).

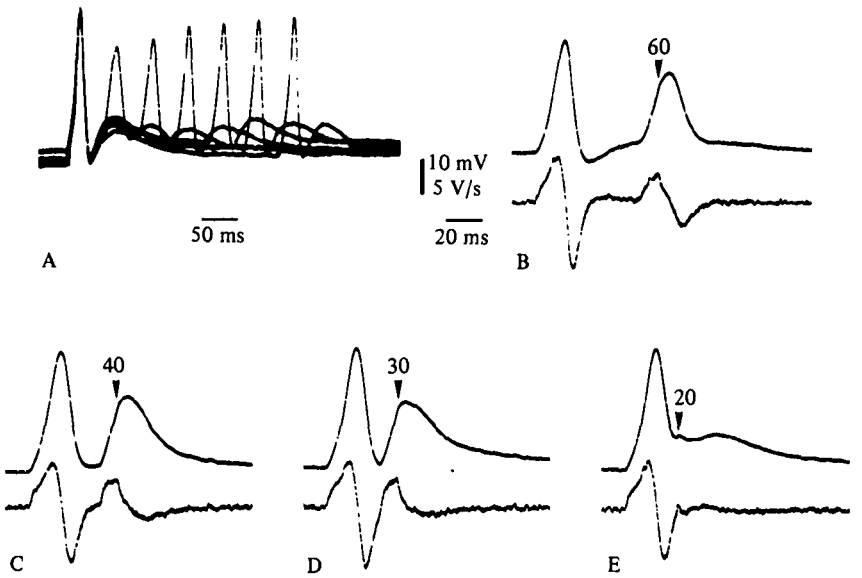

Fig. 10. Refractoriness of the action potential. (A) Superimposed recordings of the regenerative membrane responses to 10 ms stimulus currents separated by intervals of 50–300 ms. The relative refractoriness of excitability appears to be limited to 100–150 ms, but action potentials rose to original amplitude not before time intervals of 300 ms along with slow decay of after-depolarizations. (B–E) membrane responses with stimulus intervals between 20 and 60 ms. Arrowheads mark end of second stimulus pulse, number above arrowhead gives stimulus interval. The absolute refractoriness fell within less than 30 ms stimulus interval (see D, E).

In analogy to *Miamiensis* it is likely that active osmotic and ionic regulation of the cytoplasm also occurs by contractile vacuoles in *Paramecium calkinsi*. In contrast to invertebrate axons, however, where the spike mechanism is due to a Na^+-electrogenesis, the spike mechanism in this marine ciliate, like in freshwater ciliates, involves a Ca^{2+}-electrogenesis. Due to the very low intracellular Ca-activity usually found in living cells, presumably 10^{-7} M or lower, variation of the external Ca^{2+} concentration induces only comparatively small changes in the total Ca^{2+} gradient across the cell membrane. Thus, with ASW-dilutions from 100% to 20%, little influence on the action potential would be expected by the concomitant decrease in the Ca^{2+} gradient.

The Ca^{2+}-electrogenesis

The experiments described above present the following evidence for Ca^{2+} ions carrying the inward current during the action potential: (1) Removal of Ca^{2+}, but not of Mg^{2+} or Na^+, from the bathing solution reversibly abolished the electrical excitability. (2) Replacement of Ca^{2+} by Sr^{2+}, however, retained the action potential or early inward current. (3) Raising the external Ca^{2+} concentration produced an increase in amplitude and rate of rise of the action potential, and also increased the peak early inward current. The action potentials and membrane currents recorded were very similar to those shown for freshwater ciliates (for references see Machemer & de Peyer, 1977; Eckert & Brehm, 1979). In both groups, freshwater as well as marine ciliates, t[illegible]

action potentials appear to be due to an increase in membrane conductance to Ca^{2+} ions, despite the vast difference in the ionic composition of their external environment. The excess Na^+ in the fluid habitat of marine ciliates is not directly involved in the generation of action potentials, although removal of Na^+ had some drastic effects on the membrane resistance and rectification. Thus external Na^+ appears to have some indirect effects on membrane excitation by e.g. changing the I–V relationship, and by influencing the relative threshold for the generation of action potentials. It has recently been reported for the wild-type and paranoiac-mutant of the freshwater ciliate *P. tetraurelia* that Na^+ ions can carry a slow inward current, which is absent in the pawn-mutant, where the Ca^{2+}-electrogenesis is defect (Saimi & Kung, 1980). The role of external Na^+ in the marine species should therefore be more carefully investigated at various Na^+ concentrations and with different Na-substitutes under voltage-clamp conditions.

In conclusion, the present study has shown that the mechanism of electrical excitation in a marine ciliate is very similar to that shown for freshwater ciliates, and relatively unaffected by variations of ionic concentration and osmolarity of the external medium. By tolerating a wide range of external salinity, marine cells allow ample space for experimental manipulation of the external ionic concentrations. A more thorough comparison of the electrical membrane properties of freshwater and marine ciliates might provide further support for the hypothesis (Potts & Parry, 1964; Kaneshiro, Holz & Dunham, 1969) of a freshwater ancestry of brackish-water and marine ciliates.

This work was supported by the Deutsche Forschungsgemeinschaft, SFB 114, TP A5.

REFERENCES

ABBOTT, N. J. & TREHERNE, J. E. (1977). Homeostasis of the brain microenvironment: A comparative account. In *Transport of Ions and Water in Animals* (ed. B. L. Gupta, R. B. Moreton, J. L. Oshman and B. J. Wall), pp. 481–510. New York: Academic Press.

CARLSON, A. D. & TREHERNE, J. E. (1977). Ionic basis of axonal excitability in an extreme euryhaline osmoconformer, the serpulid worm *Merceriella enigmatica* (Fauvel). *J. exp. Biol.* **67**, 205–215.

DE PEYER, J. E. & MACHEMER, H. (1977). Membrane excitability in *Stylonychia*: Properties of the two-peak regenerative Ca-response. *J. comp. Physiol.* **121**, 15–32.

ECKERT, R. & BREHM, P. (1979). Ionic mechanisms of excitation in *Paramecium*. *Ann. Rev. Biophys Bioeng.* **8**, 353–383.

KANESHIRO, E. S., DUNHAM, P. B. & HOLZ, JR., G. G. (1969). Osmoregulation in a marine ciliate, *Miamiensis avidus*. I. Regulation of inorganic ions and water. *Biol. Bull. mar. biol. Lab., Woods Hole* **136**, 63–75.

KANESHIRO, E. S., HOLZ, JR., G. G. & DUNHAM, P. B. (1969). Osmoregulation in a marine ciliate, *Miamiensis avidus*. II. Regulation of intracellular free amino acids. *Biol. Bull. mar. biol. Lab., Woods Hole* **137**, 161–169.

MACHEMER, H. & DE PEYER, J. E. (1977). Swimming sensory cells: Electrical membrane parameters, receptor properties and motor control in ciliated Protozoa. *Verh. dt. zool. Ges.* **1977**, 86–110.

MACHEMER, H. & OGURA, A. (1979). Ionic conductances of membranes in ciliated and deciliated *Paramecium*. *J. Physiol., Lond.* **296**, 49–60.

NAITOH, Y. & ECKERT, R. (1968). Electrical properties of *Paramecium caudatum*: Modification by bound and free cations. *Z. vergl. Physiol.* **61**, 427–452.

NAITOH, Y. & ECKERT, R. (1972). Electrophysiology of the ciliate protozoa. In *Experiments in Physiology and Biochemistry* vol. v (ed. G. A. Kerkut), pp. 17–38. London: Academic Press.

PICHON, Y. & TREHERNE, J. E. (1976). The effects of osmotic stress on the electrical properties of the axons of a marine osmoconformer (*Maia squinado*. Brachyura: Crustacea). *J. exp. Biol.* **65**, 553–563.

POTTS, W. T. W. & PARRY, G. (1964). *Osmotic and Ionic Regulation in Animals*. 423 pp. New York: Pergamon Press.

PRUSCH, R. D. (1977). Protozoan osmotic and ionic regulation. In *Transport of Ions and Water in Animals* (ed. B. L. Gupta, R. B. Moreton, J. L. Oshman and B. J. Wall), pp. 363–377. New York: Academic Press.

SAIMI, Y. & KUNG, C. (1980). A Ca-induced Na-current in *Paramecium*. *J. exp. Biol.* **88**, 305–325.

TREHERNE, J. E. & PICHON, Y. (1978). Long-term adaptations of *Sabella* giant axons to hyposmotic stress. *J. exp. Biol.* **75**, 253–263.

TREHERNE, J. E. & SCHOFIELD, P. K. (1979). Ionic homeostasis of the brain microenvironment in insects. *Trends Neurosci.* **2**, 227–230.