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ELECTRICALLY INDUCED Ca^{2+} TRANSPORT ACROSS THE MEMBRANE OF *PARAMECIUM CAUDATUM* MEASURED BY MEANS OF FLOW-THROUGH TECHNIQUE

BORIS MARTINAC * and EILO HILDEBRAND

Institut fur Neurobiologie, Kernforschungsanlage Julich, D-5170 Julich (F.R.G.)

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Transmembrane calcium fluxes related to excitation were studied in *Paramecium caudatum*. Radioactive ($^{45}Ca^{2+}$) or inactive solution was flowed through a dense suspension of unlabelled or labelled cells, and radioactivity was monitored in the solution. The organisms were electrically stimulated by means of extracellular electrodes. As a result of stimulation Ca^{2+} uptake und release was measured. The uptake response dropped with increasing number of successive stimulation periods and increased with growing stimulus amplitude and duration. Maximum uptake was obtained at 20 V/cm and at least 60 s duration and for temperatures between 10 and 15°C. A Ca^{2+} influx of 700 pmol/1000 cells upon 1 min stimulation was measured at 15°C. This corresponds to an increment of the intraciliary [Ca^{2+}] of about $5 \cdot 10^{-4}$ M. Ca^{2+} release was dependent on the stimulus amplitude in a similar manner as was Ca^{2+} uptake. Photographic recordings of the swimming behaviour of the organisms were used to interpret the flux data. At temperatures up to 15°C the cells swam backward perpendicular to the applied electric field of 10 to 20 V/cm. At 25°C they showed forward spiralling movement. For the first time evidence was brought for stimulated Ca^{2+} influx in *Paramecium* at physiological temperatures. It is concluded from the results that a strong active Ca^{2+} extrusion from the intraciliary space counteracts the influx. The Ca^{2+} pump rate must be at least $8 \cdot 10^{12}$ calcium ions per s per cm^2 ciliary surface.

Introduction

The reversal of the ciliary stroke which leads to the so-called avoiding reaction of *Paramecium* [1] is caused by a transient elevation of the intraciliary concentration of ionized calcium [2]. Electrophysiological studies have shown that the ciliary motor response is accompanied by a rapid regenerative Ca^{2+} inward current [3,4] through voltage-sensitive channels in the ciliary membrane [5,6]. Subsequent renormalization of the ciliary stroke is consequently assumed to be the result of decreased intraciliary Ca^{2+} concentration by active Ca^{2+} extrusion [7,8] together with Ca^{2+} current inactivation [9]. Attempts have been made to directly measure Ca^{2+} translocation across the membrane of *Paramecium*. So far $^{45}Ca^{2+}$ uptake associated with excitation could be detected in *Paramecium tetraurelia* only at 0°C but not at 23°C, presumably because of a strong concomitant active Ca^{2+} extrusion [10]. Recently the influx of $^{133}Ba^{2+}$, which permeates through the voltage-sensitive channels as well, was used for tracing the action current at physiological temperatures [11].

In this paper we present Ca^{2+} transport measurements with *Paramecium caudatum* obtained by the use of a flow-through technique which enables us to detect $^{45}Ca^{2+}$ uptake associated with excitation at temperatures between 5 and 25°C, as well as $^{45}Ca^{2+}$ release from radioactively loaded cells.

* Present adress: Abteilung fur Biologie, Ruhr-Universitat, D-4630 Bochum, F.R.G.

The close relationship between the intraciliary Ca^{2+} concentration and the direction of the effective ciliary stroke [2,12] makes the swimming behaviour a readily observable indicator of the momentary Ca^{2+} concentration in the cilia. Therefore the swimming behaviour was always recorded parallel to the $^{45}Ca^{2+}$ flux measurements to monitor temporal changes of the intraciliary Ca^{2+} concentration.

Materials and Methods

Culture and preparation of cell suspension

Paramecium caudatum obtained from the Nencki Institute of Experimental Biology in Warsaw was reared in a hay infusion at room temperature. Cells were harvested approx. 2 weeks after inoculation by centrifugation at $170 \times g$ for 2–3 min, resuspended in washing solution and washed several times by use of their negatively geotactic behaviour. Cell suspensions were kept over night in the washing solution at room temperature. Suspensions were further concentrated up to $1 \cdot 10^4$ to $4 \cdot 10^4$ cells/ml by sucking off solution through a Nuclepore filter (Nuclepore Corp., Pleasenton). Cell densities were routinely determined by direct count of representative samples. The protein content, measured after Lowry et al. [13] with bovine serum albumin as a standard, was 56 μg per 1000 cells.

Chemicals and solutions

$^{45}CaCl_2$ in aqueous solution (specific acitvity approx. 2 mCi/ml) was obtained from Radiochemical Centre, Amersham. All other chemicals were of analytical grade (Merck). The washing solution contained 1 mM Tris-HCl and 10^{-4} M $CaCl_2$. The pH of all solutions was adjusted by Tris-HCl to pH 7.2. Solutions used in Ca^{2+} uptake measurements were radioactively labelled by addition of $^{45}CaCl_2$ to a final activity of $(1–3) \cdot 10^3$ cpm/nmol Ca^{2+}, as determined by liquid scintillation.

Flow-through assay

The principle of the applied method is to flow radioactively labelled or unlabelled Ca^{2+} solution through a suspension of paramecia and to measure Ca^{2+} uptake or Ca^{2+} release, respectively, by monitoring the radioactivity in the solution after it has passed the suspension.

The experimental chamber is made from plexiglass and has inner dimensions of $20 \times 10 \times 10$ mm. Two holes at the small sides, serving as inlet and outlet, are covered by 13 mm Nuclepore filters (5 μm pore size), which are fixed by Sweenex filter holders (Millipore Corp., Bedford, MA). To prevent laminar flow and to accomplish good mixing the suspension was gently stirred by a small magnet spinning around in a circular depression in the bottom of the chamber. Solution was pressed through the chamber by means of a peristaltic pump (LKB 2120 Vario Perpex Pump), and every minute fractions of ten drops (0.67 ml) were collected by a fraction collector (LKB 2112 RediRac). The total volume of the chamber including the tube connection to the fraction collector was approx. 2.5 ml. The time resolution, which is limited by the mixing of solutions of different radioactivity, was in the range of 3 to 4 min.

The temperature was kept constant by means of a feedback peltier device with an accuracy of ±0.5°C and was measured by a calibrated NTC resistor in the suspension. Radioactivity was determined in a scintillation counter (Betascint BF 5000, Berthold) after the fractions had been mixed with 5 ml scintillation fluid (Insta-Gel, Packard Instruments).

The main disadvantage of the flow-through assay is that cationic stimulation cannot be applied since all materials used in the chamber, mainly plexiglass, silicon tubes, and Nuclepore filters show more or less strong cation adsorption which causes a remarkable $^{45}Ca^{2+}$ release upon sudden increase of $[K^+]_0$.

Electrical stimulation

Two opposite sides of the chamber are covered with 20×10 mm platinum foil, separated by 10 mm, through which an external electric field could be applied. The stimulus programme which was used throughout the experiments was the same as described by Browning and Nelson [10] Trains of 10-ms square pulses of alternating polarity with an interval of 40 ms were applied. This programme was found in preliminary studies to be most effective in causing Ca^{2+} uptake by the cells. Gas bubbles at the electrodes, which would indicate electrolysis, were never observed.

Ca^{2+} uptake studies

After the chamber had been filled with a dense

non-radioactive suspension of paramecia, radioactively labelled solution was continuously passed through. After about 40 min, when a quasi steady state of radioactivity in the collected solution was reached (Fig. 1a), the first stimulation period (usually 1 min) was applied. The stimulus was usually repeated twice with an interval of 20 min. After the third stimulation cells were collected on the Nuclepore filters and, after dessiccation in a drying apparatus, radioactivity was determined. Radioactivity of the filters was determined in separate experiments without paramecia. Control experiments were carried out with and without paramecia in the absence of stimulation.

Ca^{2+} release studies

Paramecia were incubated over night in radioactively labelled solution after some μl of $^{45}CaCl_2$ had been added to a few ml of dense cell suspension. Non-radioactive solution was flowed through the chamber which contained $^{45}Ca^{2+}$ loaded cells. Stimulation periods of 1 min were applied 40 min, 60 min, and 80 min after the beginning of the experiment (Fig. 1b). Experiments without paramecia served as references to calculate Ca^{2+} release. Because of the unknown radioactive labelling of the cells only qualitative results could be obtained.

Computer evaluation

Data obtained by the flow-through experiments were plotted and evaluated with the help of a PDP 11/40 computer (Digital Equipment International Ltd., Galway). The radioactivity increase in the solution after flowing through $^{45}Ca^{2+}$ is not a single exponential function of time. A fast time course, which corresponds to the exchange of non-radioactive by radioactive solution in the flow system, can be satisfactorily described by a single exponential function with a half-time of about 3 min (Fig. 1a). Thereafter the values deviate from the curve, probably because of $^{45}Ca^{2+}/^{40}Ca^{2+}$ exchange at various parts of the chamber and by the organisms. Data points between 20 min and 40 min and at 100 min were taken for a least-square fit which was used as zero line to calculate stimulated Ca^{2+} uptake by the cells (Fig. 1a).

Behavioural registrations

The swimming behaviour of *Paramecium* was recorded by the time exposure dark field photographic technique [14]. A rectangular glass chamber (20 X 20 X 1 mm) provided with platinum electrodes at two opposite sides was used to receive the organisms. Illumination was supplied from one side by a quartz-iodine lamp (Intralux 150 H Volpi, Suisse) with light fiber optics. Photographs were taken with a conventional reflex camera.

Results

Electrically stimulated Ca^{2+} uptake

As a result of electrical stimulation a transient decrease of radioactivity in the solution was found (Fig. 1a). The response reached its maximum usually within 4 min after the onset of stimulation and relaxed within about 20 min. In the absence of paramecia no change in radioactivity could be observed. The integrated loss of radioactivity can therefore be regarded as Ca^{2+} uptake by the organisms. Ca^{2+} uptake as the result of the first stimulation period was about 500 pmol/1000 cells at 25°C. Responses to subsequent stimulation were always smaller and usually disappeared after the third stimulation period (Fig. 1a). The sum of all responses in a single experiment was approx 600 pmol/1000 cells (Temp. 25°C).

If the first stimulation was delayed, the response gradually disappeared. Also, a second response decreasing with respect to the first one was observed when the interval between the stimulation periods was increased. These results lead to the conclusion that $^{45}Ca^{2+}$ uptake is measurable only as long as the specific radioactivity of the organisms is below that of the extracellular medium.

Dependence of Ca^{2+} uptake on stimulus amplitude and duration

Ca^{2+} uptake was dependent on stimulus amplitude as shown in Fig. 2. At 4 V/cm the response was small and sometimes undetectable. With increasing amplitude the Ca^{2+} uptake increased and reached its maximum at 20 V/cm. Further increase of the stimulus caused a slight drop of the first response. At 28 V/cm and 36 V/cm the second and third response tended to

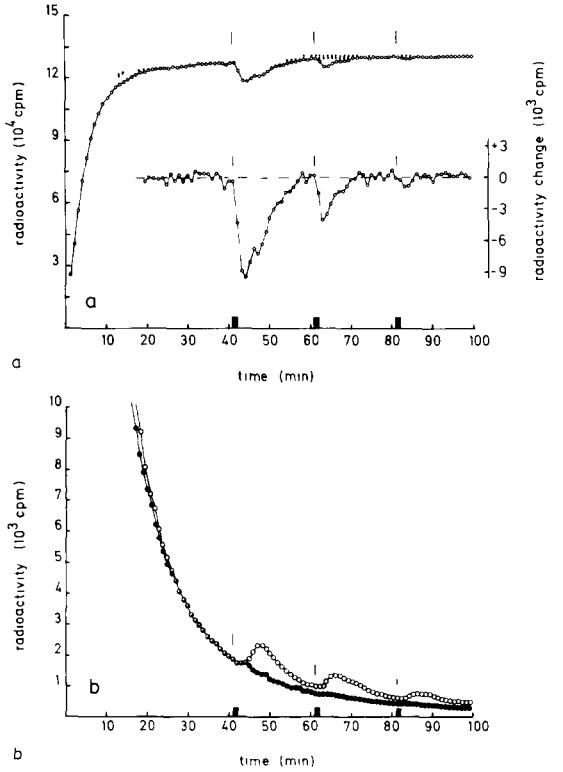

Fig. 1. (a) Single Ca^{2+} uptake experiment. Number of cells 80 000, temp. 25°C. Abscissa. Time after the beginning of flow-through $^{45}Ca^{2+}$-labelled solution. Stimulus amplitude 20 V/cm, duration 1 min (black rectangles). Radioactivity in the solution after passing the *Paramecium* suspension (open circles). Dotted curves describe fast ($t_{1/2}$ = 3 5 min) and slow ($t_{1/2}$ = 19.8 min) kinetics of the flow system. Inset Revised plot obtained after substraction of measured values from slow kinetics curve. The area below the dashed line represents $^{45}Ca^{2+}$ uptake caused by stimulation. (b) Single Ca^{2+} release experiment. Number of cells 43 000, temp. 5°C. Abscissa. Time after the beginning of flowing non-radioactive solution through suspension of $^{45}Ca^{2+}$-labelled paramecia. Stimulation as in (a). Ordinate. Radioactivity in the solution after passing the chamber. Open circles: Experiment with paramecia, solid circles: Control without cells.

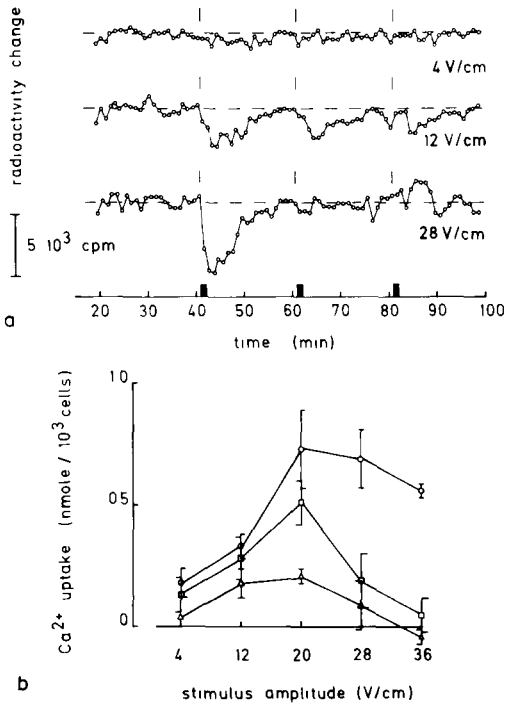

Fig. 2. Effect of stimulus amplitude on Ca^{2+} uptake. Stimulus duration 1 min, temp. 15°C. Upper part: Single experiments. Data were normalized to a specific radioactivity of $2 \cdot 10^3$ cpm/nmol Ca^{2+} and 38 000 cells. Abscissa as in Fig. 1a. Lower part: Mean values ± S.D. of the first (○), second (□), and third response (△) from three experiments, except the first response at 20 V/cm which represents the mean of 12 experiments.

disappear. At stimulus amplitudes above 36 V/cm the organisms were partly damaged and the experiments were therefore discarded. Interestingly, the net Ca^{2+} uptake at the end of the experiment was fairly constant, about 300 pmol/1000 cells, and independent of the stimulus amplitude.

Ca^{2+} uptake depends also on the duration of the stimulation period (Fig. 3). While with increasing stimulus duration the first response steadily raised up to a saturation level, the subsequent responses declined and became finally undetectable.

Temperature dependence of Ca^{2+} uptake

The results of this series are shown in Fig. 4. The first response which could be observed at all temperatures tested, showed its maximum at 10°C and declined significantly at higher temperatures. Between 10 and 25°C also the second and third response appeared. Both had their maxima at 15°C and became progressively smaller when the temperature was further increased. The net Ca^{2+} uptake at the end of the experiment showed a temperature dependence which roughly reflects that of the first response.

Ca^{2+} release

Upon electrical stimulation a transient increase of

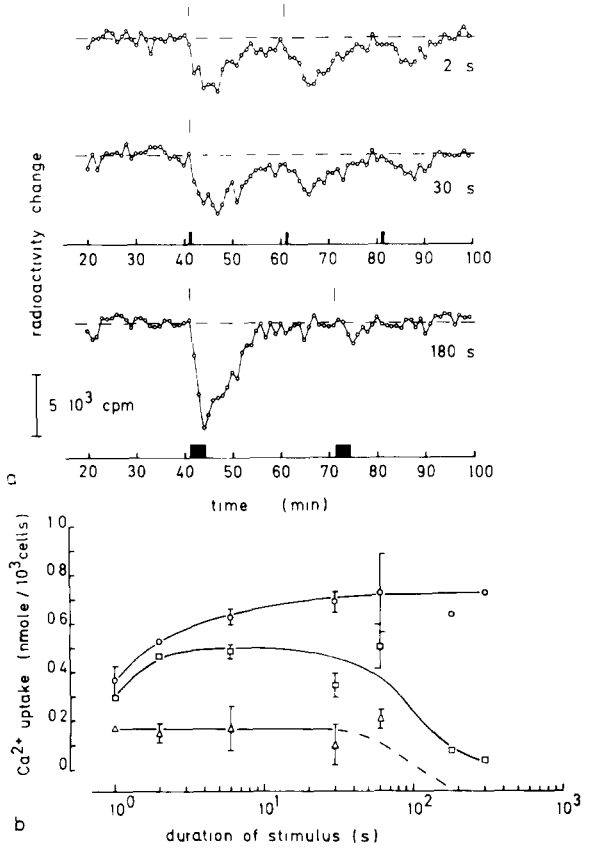

Fig 3 Effect of stimulus duration on Ca^{2+} uptake. Stimulus amplitude 20 V/cm, temp. 15°C. Upper part. Single experiments. Abscissa as in Fig. 1a. Data were normalized as in Fig 2. Lower part. Mean values ± S.D. of the first (○), second (□), and third response (△) of three experiments, except values at 180 and 300 s which represent the mean of two experiments.

radioactivity in the solution could be measured (Fig. 1b), which can be attributed to $^{45}Ca^{2+}$ release from the radioactively loaded cells. The Ca^{2+} release became generally larger when the amplitude was raised (Table I). The decrease of the third response at 36 V/cm probably results from the increased first response. The applied method does not permit calculation of the absolute amount of released Ca^{2+}.

Behaviour during electrical stimulation

Characteristic swimming traces can be distinguished on the dark-field recordings (see e.g. Fig. 5); Wavy or helical lines indicate forward movement, whereas smooth traces with a fine ripple represent

TABLE I

TOTAL (STEADY PLUS STIMULUS-INDUCED) Ca^{2+} RELEASE FROM *P. CAUDATUM* AT DIFFERENT STIMULUS LENGTH

Mean values ± S D. of three experiments. Stimulation was applied for 1 min at the 41st, 61st, and 81st min after the beginning of the experiment. Temperature, 15°C

Stimulus amplitude (V/cm)	Ca^{2+} release (rel. units)		
	During 41st–60th min	During 61st–80th min	During 81st–100th min
0	3.8 ± 2.5 [a]	2.0 ± 1.5 [a]	1.5 ± 1.3 [a]
4	4.3 ± 1.0	2.3 ± 0.5	1.5 ± 0.3
20	5.0 ± 2.0	4.3 ± 1.0	3.0 ± 0.8
36	7.8 ± 2.3	4.5 ± 1.5	2.0 ± 0.8

[a] Steady Ca^{2+} release in the absence of stimulus.

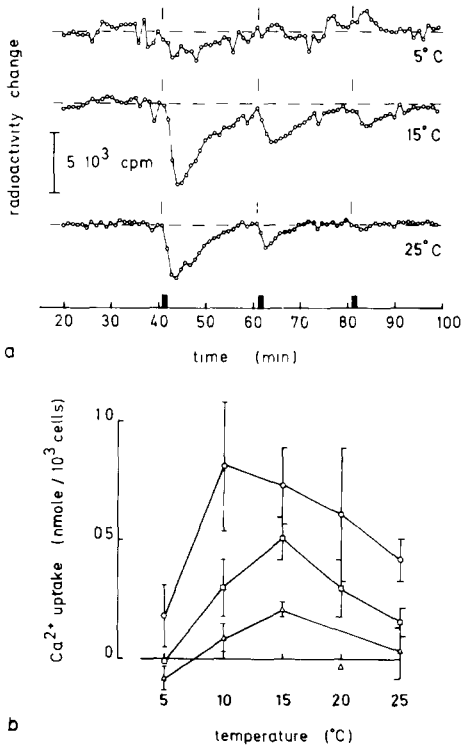

Fig. 4 Temperature dependence of electrically induced Ca^{2+} uptake. Stimulus amplitude 20 V/cm, duration 1 min. Upper part Single experiments. Abscissa as in Fig. 1a. Data were normalized as in Fig. 2. Lower part. Mean values ± S.D. of the first (○), second (□), and third response (△) of three experiments, except the first response at 15°C which represents the mean of 12 experiments.

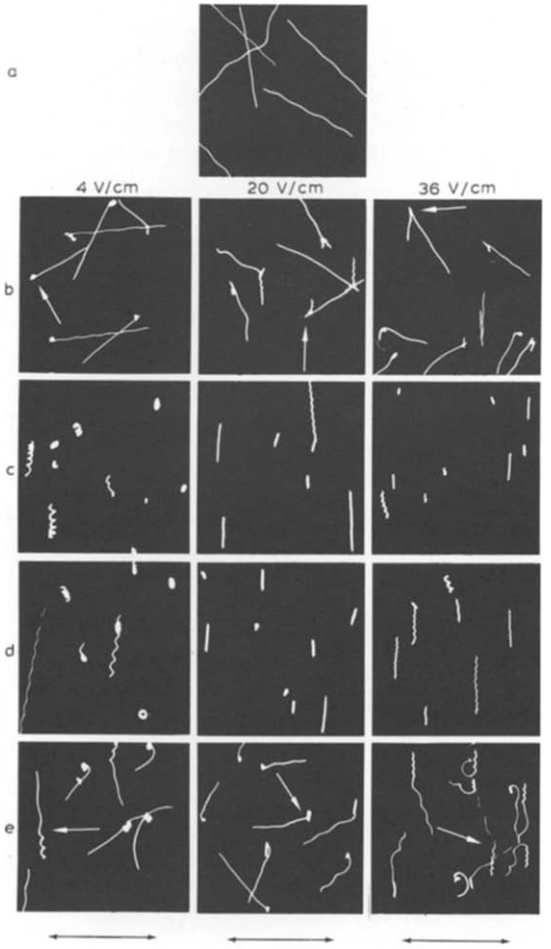

Fig. 5. Locomotor behaviour of *P. caudatum* at different stimulus amplitude. Stimulus duration 1 min; temp. 15°C. Dark-field photographic recordings, 6 s exposure. Horizontal arrows indicate the direction of the electric field and a distance of 4 mm. (a) Normal forward movement in the absence of stimulation. (b) During the onset of stimulation (arrows) 3 s after the beginning of exposure. (c) 20 s, and (d) 40 s after the onset of stimulation. (e) During removal of the stimulus (arrows) 3 s after the beginning of exposure.

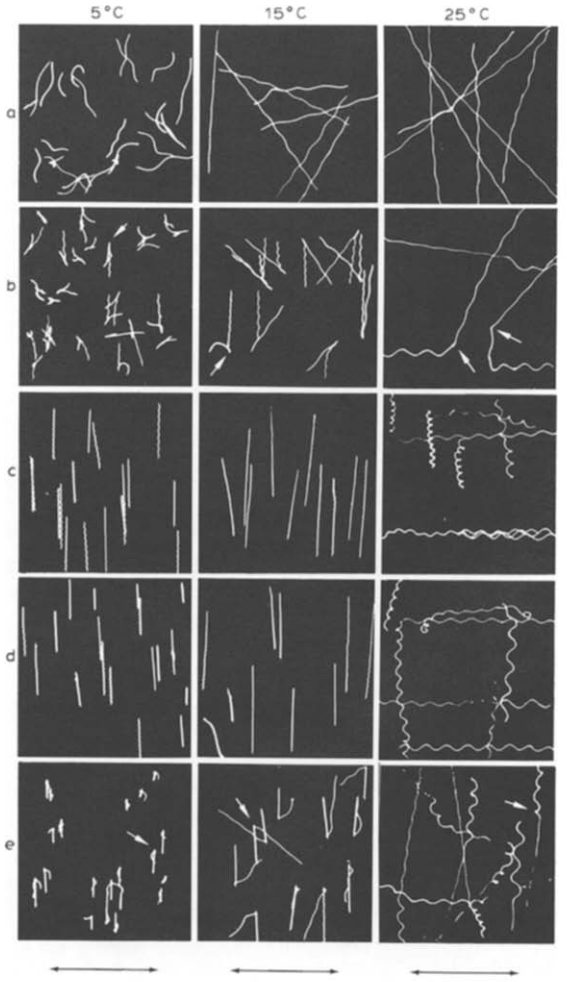

Fig. 6 Locomotor behaviour of *P. caudatum* at different temperature during electrical stimulation. Stimulus amplitude 10 V/cm; duration 1 min. Other experimental conditions as in Fig. 5.

backward swimming. Reversal of the swimming direction can often be seen when the stimulus is switched on or off (rows b and e). No difference in the motor response could be detected between the first and subsequent periods of stimulation.

The locomotor behaviour at different stimulus amplitude is shown in Fig. 5. The behaviour during stimulation with 4 V/cm was not uniform. Most of the organisms started to spin around at the place when the stimulus was switched on. Thereafter the behaviour showed some tendency to renormalize: Most of the cells swam forward on a spiral path oriented predominently perpendicular to the electric field. With stronger stimulation (20 V/cm to 36 V/cm) the cells swam backward perpendicular to the electric field until the stimulus was removed. Backward swimming was slower at 36 V/cm. It was further slowed down during stimulation, and finally the majority of cells showed slow forward spiralling.

Swimming traces at different temperatures are shown in Fig. 6. At 5 and 15°C the organisms swam

backward perpendicular to the electric field. The swimming rate was somewhat lower at 5°C and the cells slowed further down during maintained stimulation. At 25°C no reversal could be observed. The movement was forward spiralling either perpendicular or parallel to the electric field. In all experiments normal movement reappeared immediately after the stimulus was removed, but the swimming rate was slightly enhanced.

Discussion

Ca^{2+} uptake as a result of excitation

Our experiments demonstrate an electrically stimulated Ca^{2+} uptake in *Paramecium* at physiological temperatures. The applied stimulation causes the organisms to swim continuously backward, to spin around, or at least to exhibit enhanced spiralling during forward movement. This behaviour corresponds to the reorientation of the effective ciliary stroke towards the anterior end of the cell [12] and can thus be regarded as a result of excitation. It seems evident that Ca channels in the ciliary membrane, which have been postulated by various electrophysiological studies [3,4,7], must be open under these conditions permitting Ca^{2+} to flow into the cell down its electrochemical gradient. In general, the results reported in this paper confirm the Ca^{2+} current hypothesis of Eckert [7].

The swimming traces indicate that during stimulation $[Ca^{2+}]_i$ always exceeds the resting level of about $5 \cdot 10^{-8}$ M (Hildebrand, E. and Dryl, S., in preparation) and is kept fairly constant as long as the stimulus lasts (Fig. 5). This indicates a steady Ca^{2+} uptake during stimulation. The phenomenon, that the organisms swim predominantly perpendicular to the alternating electric field, was reported earlier [15] and is not yet fully understood.

With the flow-through technique Ca^{2+} uptake by paramecia can be measured only as far as the specific radioactivity in the medium is higher than in the cells. An equilibrium between intra- and extracellular radioactivity, which is reached after some time in the absence of stimulation, is probably due to some Ca^{2+} leakage across the membrane. The observation that the uptake responses decline with increasing number of stimulation periods is in line with this interpretation. An increasing first response will consequently reduce subsequent responses. Therefore the first response must be regarded as the most reliable measure. This peculiarity of the results can be readily explained by postulating a strong active Ca^{2+} efflux from the cells [10]. Under steady-state conditions $^{45}Ca^{2+}$ uptake would always be cancelled by an $^{45}Ca^{2+}$ extrusion and would thus become undetectable.

Ca^{2+} uptake increased with increasing voltage, but was slightly reduced at stimulus amplitudes of 28 V/cm and 36 V/cm (Fig. 2). Accordingly backward swimming occurred at 20 V/cm but not at 4 V/cm and was slower at 36 V/cm (Fig. 5) indicating a smaller increase of $[Ca^{2+}]_i$ at the lowest and highest voltage. The growing Ca^{2+} uptake at increasing stimulus strength is consistant with the voltage-dependent increase of Ca^{2+} conductance, (g_{Ca}) [3]. Taking into account a time constant of the membrane of about 20 ms [16] during the regenerative response, an electric field of 20 V/cm corresponds to a depolarization of about 50 mV (24 to 80 mV depending on the orientation of the cell relative to the electric field) of the membrane area facing the cathode. At stronger depolarization no further increase of g_{Ca} can be expected from the current/voltage relation [17]. The falling Ca^{2+} uptake at higher stimulus strength may be due to a partial damage of the cells.

Ca^{2+} influx and intraciliary $[Ca^{2+}]$. Quantitative considerations

The stimulus-induced Ca^{2+} uptake can be regarded as almost pure Ca^{2+} influx through the electrically excitable ciliary membrane. $^{45}Ca^{2+}/^{40}Ca^{2+}$ exchange at binding sites at the cell surface which takes place within 1 min [18] would be accomplished during the period prior to stimulation. A maximum Ca^{2+} influx of about 700 pmol/1000 cells was measured as a result of the first stimulation period of 1 min duration and 20 V/cm amplitude at 15°C. Taking into account some passive $^{45}Ca^{2+}$ uptake during the preperiod and the concomitant active Ca^{2+} efflux (see above) the actual Ca^{2+} influx would be even larger.

From the absolute refractoriness of the Ca^{2+} system of 30 ms and the relative refractory period of about 250 ms (Ref. 3, Fig. 5b) one can estimate that maximally 50% of the Ca^{2+} current will be activated by every pulse of the 10 Hz stimulation programme. The maximum Ca^{2+} uptake of one single depolarizing

pulse of 50 mV (see previous section) would be therefore about $2 \cdot 10^{-15}$ mol. Assuming a total number of 15 000 cilia per cell as an upper limit [7], a Ca^{2+} influx into a single cilium of approx. $1.5 \cdot 10^{-19}$ mol during a maximal regenerative response would result. Eckert [7], using the discharge of the membrane capacity, arrived at $3 \cdot 10^{-22}$ mol Ca^{2+} influx per cilium and 1 mV depolarization, i.e. $1.5 \cdot 10^{-20}$ mol at 50 mV depolarization, a value which he regards as the lower limit. Since this calculation neglects the regenerative nature of the Ca^{2+} current this value is certainly far too low. Recent evaluations of Machemer and Ogura [19] gave a number of 5000 to 6200 cilia per cell, which would still increase the influx per cilium.

Dividing our value of $1.5 \cdot 10^{-19}$ mol by the volume of a cilium ($3 \cdot 10^{-16}$ l [7]) an increment of $[Ca^{2+}]_i$ of about $5 \cdot 10^{-4}$ M upon a single pulse results. This value is 5-times the $[Ca^{2+}]_0$ in our experiments. Intracellular sequestration or, more probably, strong concomitant $^{40}Ca^{2+}$ extrusion must be assumed to explain this discrepancy. A $[Ca^{2+}]_i$ of $1 \cdot 10^{-4}$ M during excitation seems not improbable since Naitoh and Kaneko [2] observed the maximum rate of backward swimming with 'membrane free' models of *Paramecium* at that concentration.

The net Ca^{2+} uptake during a 100-min lasting experiment was 330 pmol/1000 cells at 15°C. Assuming a total binding capacity of the surface of $4.2 \cdot 10^{-14}$ mol per cell (calculated after [18] with the use of 56 μg dry weight per 1000 cells, see Materials and Methods) the amount of bound calcium will be 42 pmol/1000 cells. Ca^{2+} uptake through food vacuoles is less than 1 pmol/1000 cells. The residual 290 pmol/1000 cells most likely diffuse into the cell body and/or are intracellularly sequestered.

Ca^{2+} efflux

An active Ca^{2+} outward transport, which is responsible for the maintainance of the low $[Ca^{2+}]_i$ at rest and the rapid reduction after excitation, has been proposed [7]. The interpretation of our results is in line with the assumption of a strong, steady Ca^{2+} efflux from the ciliary space [10]. Moreover, we found that $^{45}Ca^{2+}$ release from preloaded paramecia is electrically stimulated (Fig. 1b). This may reflect for the most part $^{45}Ca^{2+}/^{40}Ca^{2+}$ exchange in the ciliary space. However, also stimulated Ca^{2+} release from membrane binding sites and/or voltage-dependent activation of the Ca^{2+} pump [20] must be taken into account.

The net Ca^{2+} accumulation of 290 pmol/1000 cells during 100 min, which was found even in the absence of stimulation, is negligible as compared to the Ca^{2+} influx of about 700 pmol/1000 cells during 1 min stimulation. Thus practically all Ca^{2+} which enters the cilia during excitation is pumped out. Since backward swimming of the organisms is terminated immediately after the stimulus is removed, 700 pmol/1000 cells must be extruded within 1 min. Under the assumption that the Ca^{2+} pump is evenly distributed over the ciliary membrane and taking $6 \cdot 10^{-8}$ cm^2 for the surface area of one cilium and a total number of 15 000 cilia [7] a pumping rate of at least $8 \cdot 10^{12}$ calcium ions per s per cm^2 would be required.

Temperature dependence of Ca^{2+} transport and of behaviour

For passive Ca^{2+} diffusion through membrane channels one would expect a temperature coefficient, Q_{10}, of 1.1 to 1.3. In contrast to that a 4-fold rise of Ca^{2+} uptake between 5°C and 10°C and a maximum between 10°C and 15°C was found (Fig. 4). It could be argued that paramecia may carry out avoiding reactions, i.e., short-lasting ciliary reversals, when subjected to temperature changes, and therefore some $^{45}Ca^{2+}/^{40}Ca^{2+}$ exchange in the cell during the preperiod might reduce the measured Ca^{2+} uptake. However, (1) this would not explain the maximum uptake near 10°C, because the organisms were kept between 20 and 25°C prior to the experiments. (2) The temperature change took place within more than 40 min, and (3) the swimming traces did not show an increased number of avoiding reactions during temperature adjustment.

An explanation of the temperature dependence can be given with the help of electrophysiological observations and behavioural studies. Above 10°C the early inward current is completed within less than 10 ms, i.e., during the applied depolarizing pulse. The temperature dependence of the gating process would not influence the Ca^{2+} uptake significantly. Below 10°C, however, the early inward current is slowed down (Martinac, B., unpublished results). The number of Ca^{2+} channels which are opened during the pulse will be reduced and consequently

Ca^{2+} influx will be diminished.

The continuous backward swimming at 5 and 15°C (Fig. 6) shows that Ca^{2+} interacts with the axoneme. We assume that this is the main reason for $^{45}Ca^{2+}/^{40}Ca^{2+}$ exchange in the cilium. It is expected that active Ca^{2+} extrusion is strongly reduced ($Q_{10} \geqslant 3$) at 5°C. At low temperatures also the K^+-induced continuous ciliary reversal is prolonged [21] most probably due to an inhibition of the Ca^{2+} pump [8]. The maintained high $[Ca^{2+}]_i$ will probably diminish the E.M.F. for Ca^{2+} entry and thereby further reduce Ca^{2+} influx additionally to the retarded gating process.

The behaviour at 25°C indicates less interaction of Ca^{2+} with the axoneme. Obviously the Ca^{2+} pump activity is so much enhanced that a large amount of $^{45}Ca^{2+}$ is extruded before exchange with intraciliary $^{40}Ca^{2+}$ can take place. Additionally, a larger amount of Ca^{2+} may be exchanged during the preperiod due to enhanced membrane current fluctuations.

In summary, we conclude that the temperature dependence of Ca^{2+} uptake in our experiments is mainly determined by the high temperature coefficient of the gating kinetics and of the Ca^{2+} pump activity.

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