OSCILLATION IN *NITELLA* CELL MEMBRANE POTENTIAL AND ITS EFFECT ON CYTOPLASMIC STREAMING

A. K. SINGH*† and M. AMIN**
School of Life Sciences, Jawaharlal Nehru University, New Delhi 110 067, India.
*Present addresses: *Department of Zoology, Jagdam College, Chapra 841 301, India.*
***Centre for Biosciences, Faculty of Natural Sciences, Jamia Millia Islamia, New Delhi 110 025, India.*

ABSTRACT

Oscillations in membrane potential in *Nitella* cells, distinguishable from action potential in their polarity, amplitude and frequency, can be induced by step-wise changes in the pH of the bathing medium. These oscillations are accompanied by cessation of cytoplasmic streaming. These results indicate a coupling of cytoplasmic streaming with membrane phenomena.

INTRODUCTION

EXCITABILITY in giant algal cells is a well-known phenomenon[1] that appears to be similar to that displayed by neuron and muscle cells. In the case of algal cells it is not yet resolved whether the change in the polarity of membrane potential (Vm) is brought about by Cl^- efflux or by Ca^{2+} influx. One view[2-4] suggests that action potential (AP) is caused by increase in permeability of the plasmalemma to Cl^-, while according to another hypothesis[5,6] the plasma membrane becomes permeable to Ca^{2+} rather than Cl^- and the peak value of AP relates to the electrochemical equilibrium potential for Ca^{2+}. AP are triggered by membrane depolarization, usually through electrical pulses. In the present study we report the occurrence of oscillations in Vm without a change in the polarity of membrane potential. These oscillations can be induced in the presence of raised levels of K^+ by changing the pH of the bathing medium towards alkaline pH. Since there is cessation of streaming during the oscillation and cessation of streaming is already known to occur owing to influx of Ca^{2+}, we have analysed the roles of Cl^- and Ca^{2+} in the light of our experimental results.

MATERIALS AND METHODS

A culture of *Nitella* obtained from a perennial pond in the Jawaharlal Nehru University campus, New Delhi, was maintained in our laboratory. Prior to experimentation, large and healthy internodes (about 3 cm long) were cut from the plant and kept in artificial pond water (APW) for 24 h. APW consisted[7] of 0.1 mM each of KCl and $CaCl_2$. The following pH and K^+ concentrations of the bathing solution were employed:

(i) pH: 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 7.5, 8.0, 8.5, 9.0, 10.0, 10.5; (ii) K^+ concentration (mM): 0.01, 0.1, 0.5, 1, 2, 3, 4, 5, and 10, at different pH, given above in (i).

The pH was adjusted using KOH and HCl. Only freshly made solutions were used in the experiments. The internodal cell was mounted in a shallow chamber made of glass base with glass capillaries glued along the margins to form side walls, leaving a small gap for the outlet of the bathing solution. New solutions were introduced using a dropper and the cell was flushed repeatedly with the test solution. The algal cell in the chamber was viewed under a microscope (Leitz–Laborlux-2, Wetzler, West Germany). For electrophysiological recording of the Vm, glass capillaries (Kwikfil WPI, USA) of 1 to 1.2 mm diameter drawn by a vertical pipette puller (David Kopf, USA) were used. These were filled with 3 M KCl solution and were fitted to an electrode holder (WPI, MEH-13). The latter was connected to the input of an electrode probe amplifier (WPI, M-701). The electrode was inserted in the cell using a Leitz–Laborlux-2 micromanipulator. The indifferent electrode consisted of a capillary filled with 3 M KCl agar dipping in the bathing medium and was connected to the earth of the electrode probe. The oscillations were observed on a storage oscilloscope (Tektronix 5113) and recordings were taken on a strip chart recorder (LKB, Sweden). The velocity of streaming was determined using a stop-watch (SGA, Switzerland) by measuring the time taken by a

†For correspondence.

chosen, medium-size cytoplasmic particle to traverse eight divisions of the ocular scale, corresponding to 0.58 mm of the stage micrometer.

RESULTS

Resting membrane potential values for *Nitella* cell in APW (pH 7.0) were typically −180 mV and the normal velocity of cytoplasmic streaming was about 100 $\mu m\, s^{-1}$. Electrode insertion decreased the velocity of streaming by about half of its original value. Vm was influenced by external K^+; it changed in accordance with the Nernst equation in the range of K^+_{ext} between 0.5 and 10 mM, but K^+_{ext} had very little influence below 0.1 mM. Systematic depolarization was measured when the bathing solution was exchanged for another APW at lower pH (figure 1). Very sharp depolarization occurred in the pH range 5.0 to 4.0. On the alkaline side the Vm initially hyperpolarized to values reaching up to −200 mV. Between pH 8.0 and 10.5 a sharp depolarization was recorded; the value of pH beyond which depolarization occurred varied from cell to cell. The velocity of streaming was fairly steady in the middle of the pH range; abrupt cessation of the streaming occurred simultaneously with large depolarization of Vm at extreme high and low pH. Oscillations were observed when pH was changed towards more alkaline values in steps of 0.5 pH in those cases where Vm was significantly hyperpolarized, i.e. nearly −200 mV. It could, therefore, be expected that the step-wise change in pH towards a high value would depolarize the membrane by step changes. In a few cases change from high pH also led to oscillations. Here too the change in pH is expected to cause a relative depolarization in Vm. The oscillations, a representative sample of which is shown in figure 2, had the following characteristic features:

The Vm, at values between −180 mV and −200 mV, showed an early phase of slow depolarization and then jumped maximally up to zero, returning in the first instance to values ranging from −100 to −60 mV and then oscillating between the last two extreme values with decreasing amplitude and frequency. The mid-point of oscillation was in the range −100 to −40 mV. The first oscillation occurred within a few seconds. The total duration of oscillation was about 10 min.

The velocity of streaming remained constant in the pH range 5.0 to 8.0. It declined rapidly at both lower and higher pH and halted when the membrane depolarized sharply at extreme pH. Cessation of streaming occurred at the very onset of oscillation. When the amplitude of oscillation tapered off to below −5 mV (after about 10 min), the cytoplasmic particles started moving, though very slowly. The initial Vm values could be obtained by changing the bathing medium back to APW; the velocity of streaming, however, took hours to return to its initial value.

DISCUSSION

The membrane potential of *Nitella* and indeed of cells of a large number of plants has been found to be the sum of the passive diffusion term and an electrogenic term[8]. The latter arises from the activity of proton pumps[9] energized by ATP hydrolysis. Our

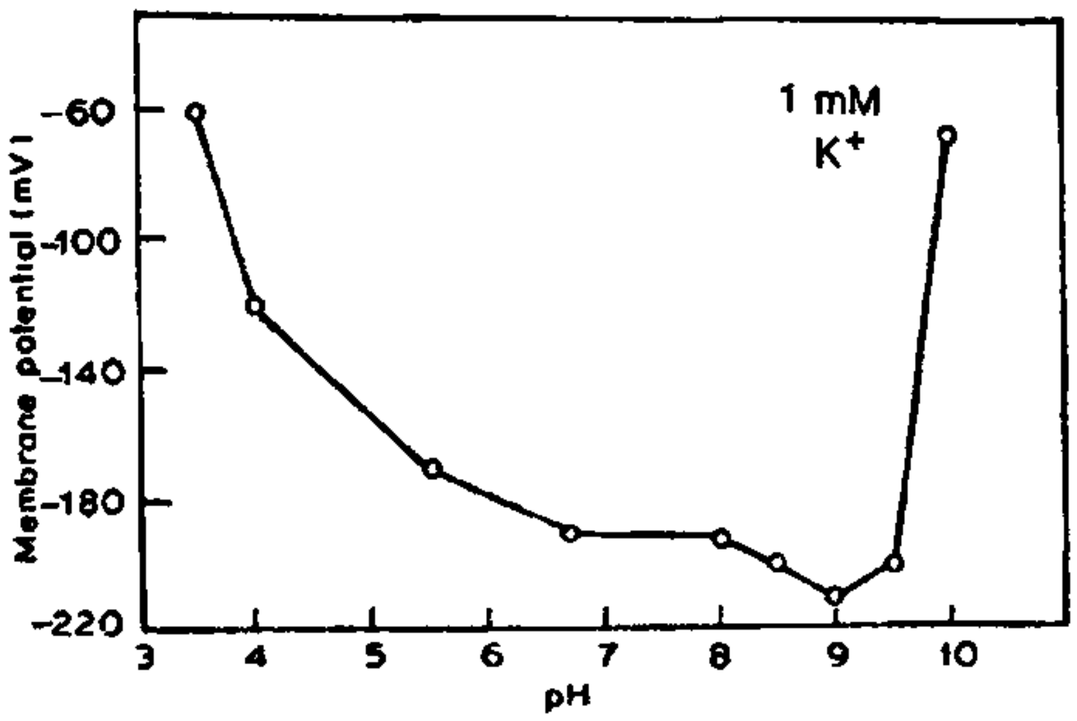

Figure 1. A typical graph showing the dependence of Vm of *Nitella* internodal cell on extracellular pH at fixed (0.1 mM) K^+ concentration.

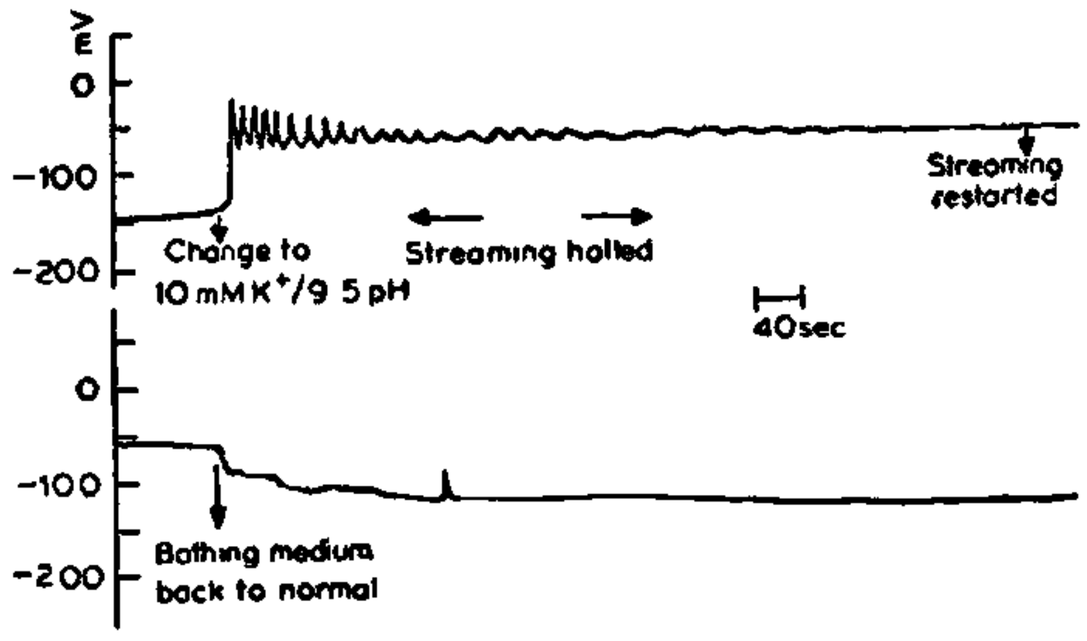

Figure 2. Oscillation in Vm of *Nitella* cell after changing the external bathing medium. The streaming remains halted during the oscillation. The lower diagram is the continuation of the upper one.

result, that high pH of the bathing medium hyperpolarizes Vm and pH below neutral depolarizes Vm, corroborates these facts since the proton efflux pump would be sensitive to pH differences across the plasmalemma. The dependence of Vm on K^+_{ext} recorded by us follows, more or less, the pattern of Vm of neurons. For relatively high concentration of KCl in the bathing medium, Vm depends on K^+_{ext} in accordance with the Nernst diffusion potential for K^+. Below 0.5 mM KCl the Vm is much less sensitive to K^+_{ext}.

The genesis of oscillations in Vm can be interpreted along the same lines that explain the phenomenon of AP in giant algal cells. Voltage-gated Cl^- channels open when the membrane is depolarized, permitting an efflux of Cl^- ions, till the Vm has reached the peak value, close to the Cl^- ion Nernst potential value. Inactivation of the channels of Cl^- flux and increased permeability to K^+ brings Vm back to the value of the resting potential as determined by the Nernst diffusion potential for K^+. The latter quantity is, for APW, close to -180 mV, which is below the threshold of excitability. By increasing the extracellular KCl concentration in our experiments we have altered two parameters of excitability, viz. the peak value of depolarization and the value of repolarization, pertaining, respectively, to Cl^-_{ext} and K^+_{ext}. While increasing Cl^- to values above 1 mM we have brought down the peak potential to zero and below zero. At the same time maximum reversal of polarity at high K^+_{ext}, is much above the resting potential. The genesis of oscillations could be that the repolarization due to K^+ efflux at the higher concentration of KCl used by us happens to be above the threshold of excitability but below that value of Vm at which the Cl^- channels are reactivated. The peak values of oscillation are reduced because of high Cl^-_{ext}.

The above explanation for the observed oscillation is on the lines of the hypothesis that in *Nitella* membrane potential can be explained in terms of Cl^- and K^+ voltage-gated channels. Our concurrent measurement of cytoplasmic streaming shows that the matter is not so simple. We observe that oscillations cause cessation of streaming during the period of oscillations and that the resumption of normal streaming needs several hours after the bathing medium has been changed to APW. While the intracellular Cl^- concentration is not known to affect cytoplasmic streaming, a change of Ca^{2+}_{int} to above 5×10^{-4} M brings about cessation of streaming in intact *Nitella* cells[10]. We are therefore forced to envisage an influx of Ca^{2+} down its electrochemical gradient into the cell interior across the plasmalemma during the oscillations. On the other hand, we cannot fully favour the hypothesis that Ca^{2+} plays the role of Na^+ in *Nitella*. Since we have not changed the extracellular Ca^{2+} concentration in our experiments, if the peak of the AP would correspond to the Ca^{2+} diffusion potential we should have obtained a constant value of peak potentials on the positive side. Therefore Ca^{2+} cannot be the only ion whose concentration gradient across the plasmalemma determines the peak of the AP[5,6]. We conclude that during AP, of which the oscillations we have observed are a special form, both Cl^- and Ca^{2+} ions are involved.

Our observation, upon reverting to APW after the period of oscillations, that the streaming velocity returned to its normal value after many hours while Vm came to its resting value in a few minutes, also indicates the involvement of Ca^{2+} in oscillation of Vm and in the cessation of streaming. Extrusion of Ca^{2+} from cytosol by Ca^{2+} efflux pumps is, presumably, a slow process. Neither Ca^{2+} active transport nor a Ca^{2+} sequestering mechanism that may be present in *Nitella* cells has been as yet reported, although evidences for the operation of both these mechanisms have been reported in higher plants[11,12].

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ANNOUNCEMENT

IGCP-274: COASTAL EVOLUTION IN THE QUATERNARY

The Indian National Committee (INC) for the International Geological Correlation Programme (IGCP) has approved Indian participation in Project-274 on 'Coastal evolution in the Quaternary'.

The inaugural meeting of the Indian National Working Group held at the Geological Survey of India, Jaipur, on 12 and 13 April 1989 was attended by specialists drawn from the Kerala University and Centre for Earth Science Studies, Trivandrum; Physical Research Laboratory, Ahmedabad; Oil and Natural Gas Commission, Dehradun and Bombay; Lucknow University; University of Delhi and Jawaharlal Nehru University, New Delhi; Sivaji University, Solapur; and the Geological Survey of India. Current and recent studies on coastal Quaternary of India being pursued by various institutions and universities were reviewed.

Considering the international aims of Project-274, the following objectives of the Indian National Working Group were accepted:

i) to document Quaternary coastal evolution of various Indian coastal types;
ii) to promote studies along the coastal zones of India, especially along (a) Rann of Kachchh (Kutch), Gulf of Khambat (Cambay) and beaches of south Gujarat; (b) cliffed shorelines of Maharashtra; (c) beaches, lagoons, mud-flats and drowned valleys of Kerala and Tamil Nadu coasts; (d) Krishna–Godavari Delta and Chilka Lake bar-lagoon complex.
iii) to promote studies on Quaternary sea level changes, neotectonics and related studies of the coastline of India.

It was also suggested at the meeting that

i) an atlas of Quaternary geomorphological, geological and allied thematic maps of the entire coastal zone of India be compiled,
ii) a geological map of the shelf region of India be compiled,
iii) a centralized database be established,
iv) a bibliography of the Quaternary of the Indian coast be periodically upgraded and published,
v) on the basis of the database already available, suitable transects be selected and studied,
vi) tidal gauge coverage for sea level changes and geodetic surveys be done, and
vii) up-to-date information on the status of knowledge on the Quaternary of the Indian coast be collected and made available to researchers.

A four-year programme (1989-1992), including field meetings and workshops on evolution of East and West coasts of India during the Quaternary and modern coastal processes, has been chalked out. The next field meeting is scheduled at Porbandar/Ahmedabad during 7 to 10 November 1989. For further details, researchers interested in the advancement of the cause of the Project may get in touch with either Dr U. B. Mathur, Director, Geological Survey of India, and Convener, NWG for Project-274, B-201 Rajendra Marg, Bapu Nagar, Jaipur 302 015, or Prof. R. Krishnanath, Co-Convener, NWG for IGCP Project-274, Reader, University of Kerala, Kariavattom, Trivandrum 695 581.