Short communication

The Role of Calcium in the Generation of Membrane Potential Oscillations in *Nitella* Cells

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Characean cells are excitable and capable of generating action potentials in response to different stimuli. These transients have been shown to be complex due to the frequent participation of both the plasmalemma and the tonoplast in their appearance. Voltage clamp experiments demonstrated the requirement for calcium in the external medium for the excitation process, also its effect on the amplitude of the spike (Hope 1961; Findlay and Hope 1964).

Besides individual action potentials, usually induced by current pulses, these cells can generate repetitive action potentials, i.e. membrane potential oscillations. The causes responsible for such oscillations and their physiological role are not completely understood. In this communication we give our results on the study of oscillations and their dependence on the presence of calcium ions in the external medium.

For this work, we used internodal cells of *Nitella mucronata*, cultured as described previously (Radenović and Vučinić 1976). The cells were kept for 24 h preceeding the experiment in a 5 mmol·l^{-1} solution of NaCl so as to remove the calcium from the cell wall; subsequently, they were placed in the standard experimental solution (1 mmol·l^{-1} NaCl + 0.1 KCl mmol·l^{-1}; pH 7.5 kept constant with a 2 mmol·l^{-1} MES/Tris buffer combination). The cells were mounted into a microelectrode chamber perfused with a solution at a rate of 5 ml·min^{-1}. Measurements of the vacuolar membrane potential were performed using the standard microelectrode technique.

In addition to the above standard experimental solution we used a solution containing additionally 0.25 mmol·l^{-1} $CaCl_2$ and another with 1×10^{-6} mmol·l^{-1} 3-(3,4-dichlorphenyl)-1,1-dimethylurea (DCMU). The cells were illuminated with a tungsten projector lamp (150 W), the light intensity on the surface of the chamber being 10 W·m^{-2}. All the experiments were performed at room temperature (25 °C).

In our studies of the excitability phenomena of plant cells were facing the problem of inducing membrane potential oscillations at will. In our previous article (Vučinić et al. 1978) we reported the probability of oscillations occurring upon impalement or "spontaneously" being approximately 10 % (of over 400 performed experiments). Those experiments were performed in a calcium free solution, but no systematic effort was made to exclude calcium from the cell wall. In a large number of experiments performed on cells in the presence of calcium, practically no oscillations could be observed (over 500 cells impaled), except for the occurrence of occasional individual action potentials.

By washing the cells with 5 mol·l^{-1} NaCl solution preceeding the experiment, we managed to increase the probability of the occurrence of oscillations to 40—50 %. Such cells exhibited oscillations under a variety of conditions: immidiately upon impalement of the cell, upon addition of DCMU into external solution, upon illumination of a dark adapted cell, etc.

In the case od oscillations occurring following the impalement of the cell, usually lasting for relatively long periods of time (see Fig. 1), the addition of calcium in the external medium stopped the oscillations.

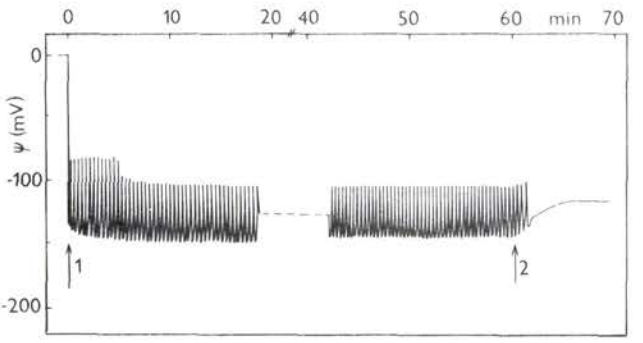

Fig. 1. An example of an oscillation of the vacuolar potential of *Nitella mucronata* occurring upon impalement by the microelectrode (arrow 1). Arrow 2 indicates the moment of addition of 0.25 mmol·l^{-1} $CaCl_2$.

By using cells washed of calcium and working in calcium-free external solution, oscillations could be induced by a number of treatments, their common denominator being the depolarization of the membrane potential above a certain threshold level. Thus, addition of DCMU to the external medium induced oscillation in the period of depolarization following immediately after the primary hyperpolarization (Fig. 2*a*). Oscillations of the membrane potential induced by light were of short duration and appeared in the period of primary depolarization

of the membrane potential (Fig. 3). Addition of calcium had a similar inhibitory effect (Fig. 2*b*).

Whenever an oscillation of the membrane potential occurred, cyclosis ceased during the first spike in the series, and the cytoplasm remained stationary until the end of the oscillation if the frequency of generation of spikes was high. Streaming resumed gradually, one to two minutes were needed for the first visible signs of cytoplasmic movement, and normal streaming rates of approximalety 60 $\mu m \cdot s^{-1}$ was restored within more than four minutes. If the period between two consecutive spikes in the oscillation was sufficiently long, streaming began again, but the succeeding spikes halted streaming repeatedly. Thus the spikes had a similar effect on cyclosis as individual action potentials induced by electrical stimuli (Hill 1941; Barry 1969). However, in this instance they were self generating and as there was no calcium present in the external medium, calcium released from internal reserves seems to be involved.

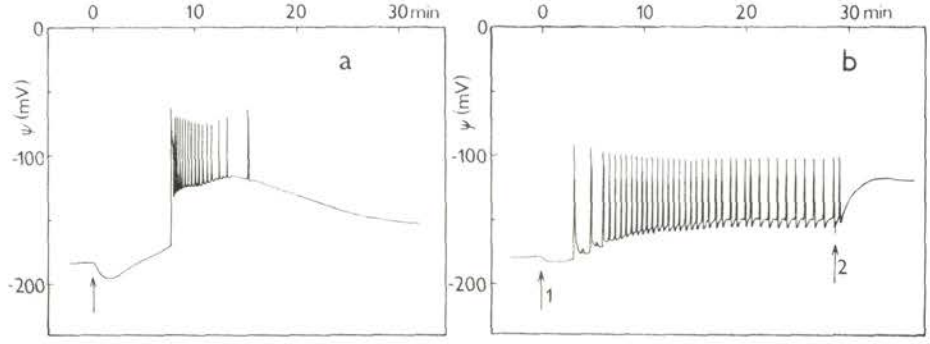

Fig. 2. Oscillations induced by the addition of 1 $\mu mol \cdot l^{-1}$ DCMU (the addition marked by arrow in example shown in 2*a* and arrow 1 in example 2*b*) Arrow 2 in Figure 2*b* indicates the addition of 0.25 $mmol \cdot l^{-1}$ $CaCl_2$.

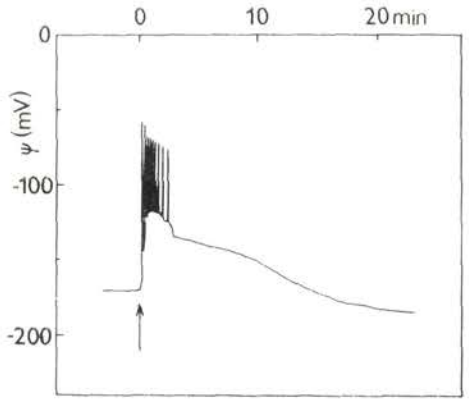

Fig. 3. Oscillations induced by illuminating a dark adapted *Nitella* cell (the start of illumination marked by arrow).

Characean cells exhibit the phenomenon of repetitive action potentials, i.e. membrane potential oscillations under a variety of conditions. In general, membrane potential oscillations can be viewed as a result of system instability in the vicinity of some critical point. In previous reports (Kishimoto 1966; Radenović et al. 1968; Vučinić et al. 1978; Keifer and Lucas 1982), as well as in this one, it has been observed that membrane potential oscillations occur mainly in the absence of external calcium. Thus absence of calcium could bring the system into unstable state. These results are in agreement with the results reported on the squid axon (Inoue et al. 1973) that show that there exists a critical value of the ratio of Ca/Na concentrations at which oscillations occur. It has also been shown that oscillations of the membrane potential can occur in *Nitella* cells if the resting membrane potential is gradually approaching the excitability threshold (Hyashi and Hirakawa 1980).

In our experiments, membrane potential oscillations occurred upon illumination of dark-adapted cells, as well as during the depolarization of the membrane potential in the presence of DCMU. Oscillations of the membrane potential immidiately after impalement also occured if the cell was in the depolarized state (when presumably the electrogenic pump ceased functioning). Common to all of them is the occurrence of oscillations in the depolarized state (indicating the involvement of a threshold). The mechanism through which depletion of external calcium induces oscillations remains to be resolved. Removal of external calcium increases oscillations remains to be resolved. Removal of external calcium increases the excitability of the cell, thereby rendering it susceptible to other factors which can cause a change in permeability and depolarize the membrane (Keifer and Lucas 1982). This could be the reason for the liberation of calcium from intracellular reserves. As a result, internal free-calcium concentration increases, similar to the case of action potentials induced by current (Williamson and Ashley 1982). In agreement with this is the observation that cyclosis ceases during the oscillation, and it has been well documented that cyclosis is greatly dependent on calcium concentration in the cytoplasm (Barry 1969).

This could mean that in non-physiological conditions of absence of calcium from the external medium, a perturbation of the cytoplasmic ionic equilibrium is inevitable. Resulting membrane potential oscillations could represent an attempt of the cell to regulate its ionic composition, during which calcium could play the role of a mediator.

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