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Radio-Frequency Rectification in Electrogenic and Nonelectrogenic Cells of *Chara* and *Nitella*

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Summary. Electrogenic cells of *Chara braunii* and *Nitella flexilis* were placed in a pulse-modulated radio-frequency electric field of up to 6000 V/m. Their vacuolar resting potentials were found to experience submillivolt *de*polarizing offsets (typically 140 μV at 250 kHz) which were relatively independent of temperature, increased linearly with resting potential from a zero near −210 mV, and had a cutoff (putatively due to ion transit times) near 5 MHz. By contrast, nonelectrogenic cells experienced *hyper*polarizing offsets (typically 1100 μV at 250 kHz) which increased in magnitude with increasing temperature, were independent of resting potential, and had a transit time cutoff near 10 MHz.

The ionic mobilities inferred from these cutoff frequencies are somewhat higher than would be expected for active transport and presumably reflect passive conductance mechanisms which therefore must be presumed different for the electrogenic and nonelectrogenic states.

Key words Characeae · electrogenesis · ion flux · radio-frequency bioeffects · rectification · transit time · fusicoccin

Introduction

The existence of an electrogenic pump or pumps in plant cells has been suspected for many years because the membrane potential is often far more negative than could be accounted for by passive diffusion potentials predicted from the Goldman-Hodgkin-Katz equation (*cf.* Spanswick, 1972; Saito & Senda, 1974). In this hyperpolarized and putatively electrogenic state the cell's resting potential is relatively insensitive to external cations and anions (e.g., Pickard, 1973), is quite sensitive to external pH (Spanswick, 1972; Saito & Senda, 1973; Roa & Pickard, 1976*b*), and has come to be attributed to electrogenic H^+ extrusion or OH^- uptake (e.g., Poole, 1978; Walker, 1980).

Electrogenic transport in plant cells has so far been investigated primarily by studying either the resting potentials and membrane resistivities of the cells or their rates of uptake of radioisotopes and by noting how these observables vary as the cells are challenged by various inhibitors or by various bathing media or by altered light intensities (*cf.* Spanswick, 1972; Poole, 1978). In particular, the only aspect of the resting potential commonly examined is its DC level although its low frequency power spectral density function (e.g., Roa & Pickard, 1976*a*; Ferrier, Morvan, Lucas & Dainty, 1979) or its response to pulsed radio-frequency irradiation (Pickard & Barsoum, 1981) are known to contain significant additional information.

In this paper the radio-frequency irradiation technique will be exploited to study the responses of electrogenic cells of *Chara* and *Nitella* to such challenges as altered resting potential, altered temperature, and fusicoccin.

Materials and Methods

General

Materials and methods were similar to those described previously Pickard & Barsoum, 1981). Unless otherwise specified, all experiments were carried out at 25 °C in a flowing electrogenic artificial pond water (EAPW) of composition (in mM): KCl 2.5, NaCl 2.5, $CaCl_2$ 0.5, $MgCl_2$ 0.5, N-2-hydroxyethylpiperazine-N-2-ethansulfonic acid (HEPES) 0.5, NaOH 0.25. Its pH at room temperature is approximately 7.35. The quantity of primary interest was the response of the resting potential of a Characean cell to a short burst of radio-frequency irradiation. At the irradiation frequencies of interest in this paper, the response was a small submillivolt offset which was separated from the DC and stochastic components of the resting potential using a digital signal averaging technique whose limit of sensitivity was approximately 1 μV.

Exposure Apparatus

The cell was placed in a 1-mm wide, EAPW-filled channel cut through a 1.6-mm thick polyacrylate sheet which was mounted to a silver ground plane. A 2-mm wide silver foil ribbon ran perpendicularly to the channel, forming a microstrip transmission line structure. One end of the cell rested under the microstrip while the other remained downstream in a relatively field-free region and was impaled by a micropipette through which the vacuolar resting potential was sensed. The root mean-square drive voltage between the microstrip and the ground plane was sensed through a high-frequency voltage probe.

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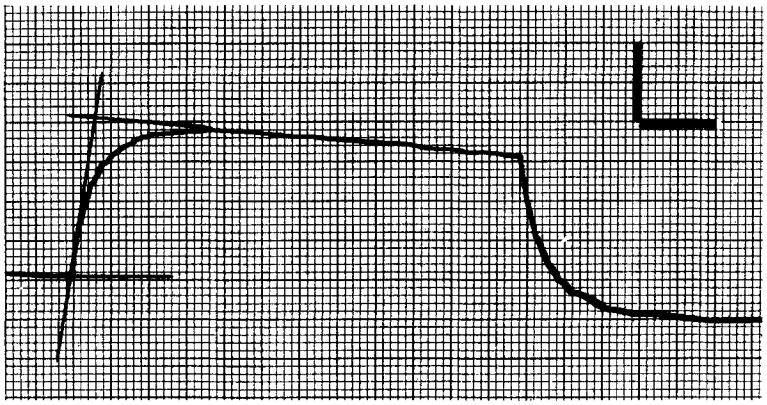

Fig. 1. Typical, 20-pulse, intracellular response curve for an electrogenic cell of *Chara braunii* showing the auxiliary lines drawn for data reduction. Pertinent data: resting potential, −153 mV; irradiation frequency, 250 kHz; drive voltage at microstrip, 4.0 V rms; temperature, 25 °C. The vertical bar is 125 μV and the horizontal bar 40 msec; hence, the offset height was 255 μV (depolarizing) and the rise-time roughly 12 msec

Table 1. Reference offsets $\tilde{V}$[mV] observed for the different cell states

State / Species	Strictly nonelectrogenic	Sometimes electrogenic		Strictly electrogenic
		Nonelectrogenic	Electrogenic	
Chara	−1.10±0.08(23)	−1.14±0.07(13)	+0.15±0.08(13)	+0.13±0.03(24)
Nitella	−1.13±0.05(43)	−1.08±0.32(2)	+0.14±0.07(2)	+0.16±0.08(5)

Data Reduction

In order to recover the true shape of the offset, which had been distorted by band-pass filtering, the data reduction method previously employed (Pickard & Barsoum, 1981) was again used as illustrated in Fig. 1: the mean slope drawn through the linear portion of the offset in the 50–250 msec range has been extrapolated back to zero time yielding a height for the undistorted pulse; the intersection of this slope with the other mean slope drawn through the initial few milliseconds of the offset determines a rise-time.

To adequately compare the data of different experiments, a normalization procedure similar to that used before (Pickard & Barsoum, 1981) has been used. Here, however, the reference offset $\tilde{V}[V]$ was taken to be that at 4 V rms microstrip drive voltage and 250 kHz, and the normalized offset is that which actually was observed divided by $\tilde{V}$ and multiplied by a correction factor to compensate for the fact that the drive voltage was not 4 V (frequency varied) or that the drive frequency was not 250 kHz (voltage varied).

The data points for each group of cells are accompanied by the notation $A = X \pm Y(Z)$ where X is the mean value of A, Y is the standard error of the mean, and Z is the number of cells in the sample.

Results

General Observations

Resting potentials of the Characean cells used in this laboratory were either less negative than −95 mV or more negative than −120 mV. The region above −95 mV can conceivably be achieved by passive diffusion mechanisms (*cf.* Pickard, 1973) and will be designated nonelectrogenic. The region below −120 mV must be due in part to active transport and will be designated electrogenic. This subsection will be concerned primarily with electrogenic cells; analogous data for nonelectrogenic cells have been reported previously (Pickard & Barsoum, 1981).

When an electrogenic Characean cell (either *Chara braunii* or *Nitella flexilis*) was impaled by the signal pipette, it was found that the measured resting potential either (*i*) jumped immediately to an electrogenic level, or (*ii*) drifted slowly from a low nonelectrogenic level to a final electrogenic state, or (*iii*) after some tens of minutes flipped from a stable nonelectrogenic state to a new stable electrogenic state. In the latter two cases, if the cells were irradiated when the resting potential was above −90 mV and the responses averaged, the offset at 250 kHz irradiation frequency and 4 V rms microstrip drive voltage was found to be *hyper*polarizing and comparable to that of normally stable nonelectrogenic cells. When, after about 20 min or more, the cells had reached their final electrogenic state with resting potentials stabilized below −120 mV, radio-frequency irradiation (250 kHz at 4 V rms) produced *de*polarizing offsets of small magnitude. Table 1 gives the mean values of the reference offsets measured for the different states of *Chara* and *Nitella*. Clearly, the state of the cell (as determined by its *present* resting potential) seems more important than either its species or the past values of its resting potential.

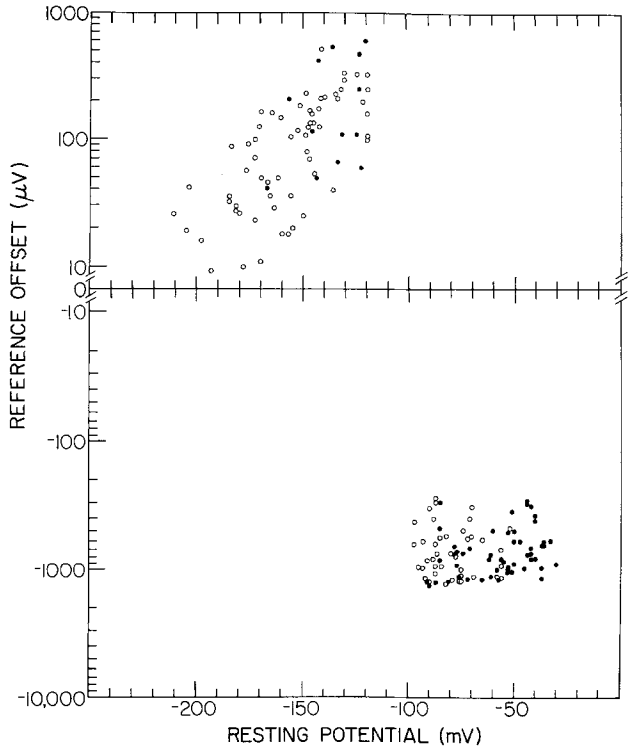

Fig. 2. A plot of the offsets (as measured at an irradiation frequency of 250 kHz and a microstrip voltage of 4.0 V rms) versus the resting potential for a number of Characean cells. The hollow symbols are for *C. braunii* and the solid symbols are for *N. flexilis*

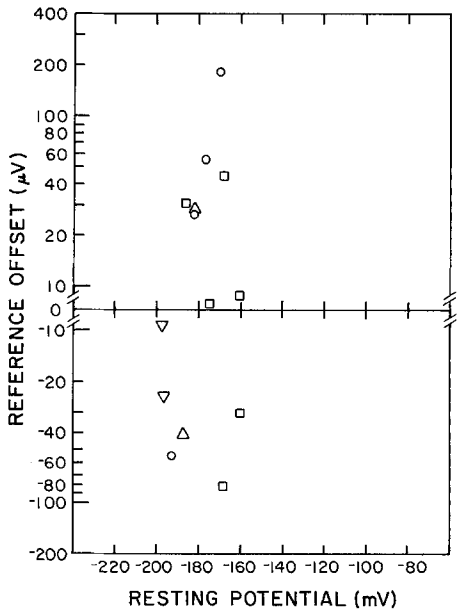

Fig. 3. A plot of the offsets ΔV (measured at an irradiation frequency of 250 kHz and a microstrip voltage of 4.0 V rms) versus the resting potential for four *Chara braunii* cells that showed a change in the sign of the offset during the course of their experiments

Figure 2 is a plot of offset versus resting potential for a large number of different cells. Clearly, the magnitude of the depolarizing offset of an electrogenic cell exhibited a variation with the resting potential (RP): at a resting potential of about −210 mV the offset was almost zero, and it grew rapidly as the resting potential became less negative. In contrast to the resting potential sensitive offset of electrogenic cells, the hyperpolarizing offset which characterizes the nonelectrogenic state is seemingly independent of resting potential. Occasionally, the resting potential of a cell would, towards the end of an experiment and without obvious provocation, drop to a nonelectrogenic level. In these cases, the offset invariably flipped over from a *de*polarization to a relatively larger *hyper*polarization. The latter was consistent in both sign and magnitude with offsets of stable nonelectrogenic cells.

The relatively wide range of resting potentials shown in Fig. 2 reflects the experimental fact observed over many years that the Characean cultures kept in this laboratory stochastically shift their electrical properties with a time scale of weeks, some periods being characterized by electrogenic cells and others by nonelectrogenic cells, both states having stable resting potentials distributed over a wide range even though the cells examined were excised from healthy appearing thalli and were streaming vigorously. In the experience of this laboratory (e.g., Pickard, 1973, or Roa & Pickard, 1976*a*), Characean resting potentials are either less negative than −90 mV or more negative than −120 mV; stable resting potentials in the band (−90, −120) are almost never encountered. With cells bathed in electrogenic artificial pond water the region above −90 mV could conceivably be achieved by passive diffusion mechanisms (*cf.* Pickard, 1973) while the region below −120 mV could be achieved only if the resting potential contained a component due to active transport.

Out of a total of some 175 cells of *Chara braunii* and *Nitella flexilis* thus far examined, seven cells of *Chara braunii* were encountered that were highly electrogenic (RP < −160 mV) and which flipped spontaneously between small depolarizing offsets and small hyperpolarizing ones. Figure 3 shows the variation of the offset at the standard reference exposure (irradiation frequency, 250 kHz; microstrip voltage, 4.0 V rms) versus the resting potential for four cells; the remaining three cells were not plotted since, although they exhibited offsets of both signs, no hyperpolarizing offsets were recorded at the standard reference exposure. This sudden change in the sign of the offset was consistently related to neither the frequency of irradiation nor the magnitude of the drive voltage, and is tentatively attributed to a hypothesized hyper-

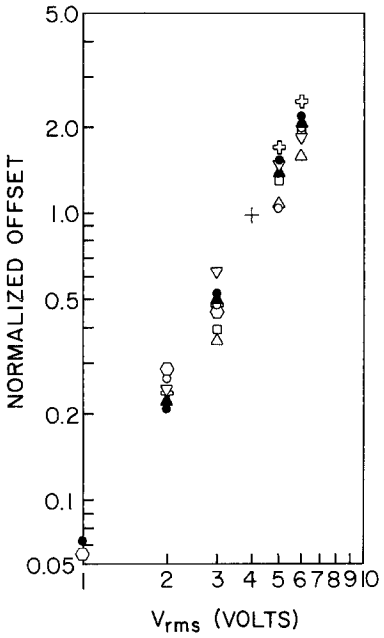

Fig. 4. Normalized intracellularly observed offset versus microstrip voltage. The hollow symbols are for *Chara braunii*; these cells had a mean $\tilde{V}$ of $+0.16 \pm 0.06(6)$ mV and a mean resting potential of $-160 \pm 7(6)$ mV. The solid symbols are for *Nitella flexilis*; these cells had a mean $\tilde{V}$ of $+0.21 \pm 0.15(2)$ mV and a resting potential of $-148 \pm 9(2)$ mV. The cross (+) denotes the normalization point. The irradiation frequency was 250 kHz

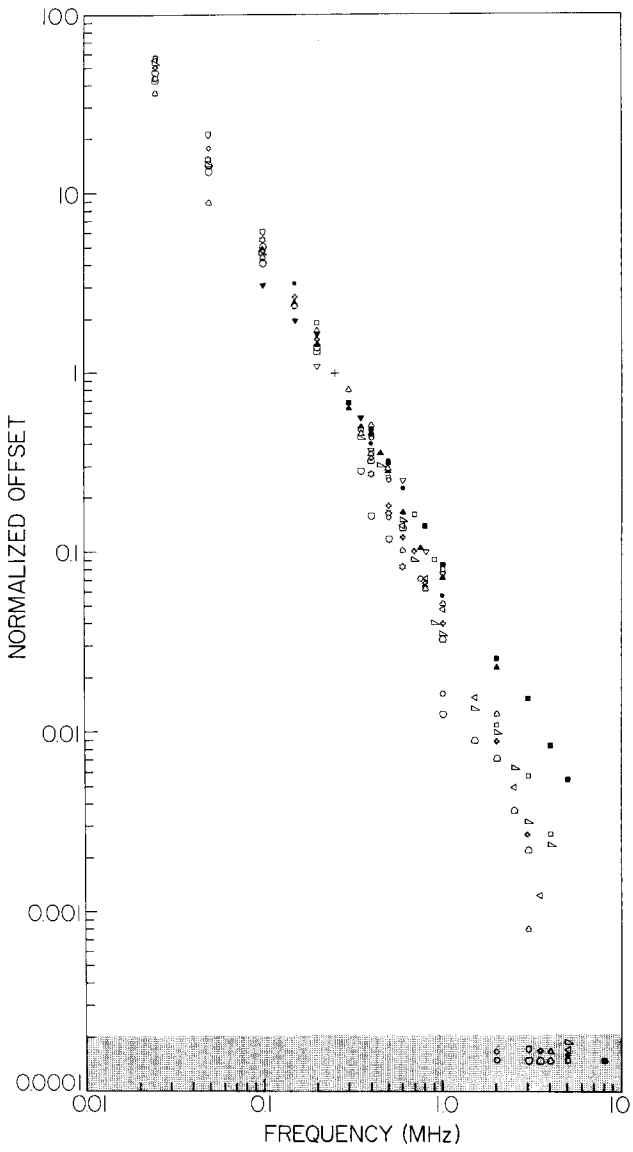

Fig. 5. Normalized intracellularly observed offset versus irradiation frequency. The hollow symbols are for *Chara braunii*; these cells had a mean reference offset of $+0.12 \pm 0.02(13)$ mV and a mean resting potential of $-150 \pm 5(13)$ mV. The solid symbols are for *Nitella flexilis*; these cells had a mean reference offset of $+0.19 \pm 0.08(4)$ mV and a mean resting potential of $-138 \pm 8(4)$ mV. The cross (+) denotes the normalization point. The shaded area at the bottom of the graph represents the noise level and the points therein represent trials in which no offset was detected and the actual offset was $\lesssim 1\,\mu V$

electrogenic state which may exist if the resting potential is more negative than -160 mV.

Figure 4 illustrates the variation of the normalized offset with applied voltage for electrogenic cells. This variation is seen to be roughly quadratic just as in the nonelectrogenic case (Pickard & Barsoum, 1981).

Figure 5 illustrates the variation of the normalized offset with frequency for electrogenic cells. The offset is seen to be roughly inversely quadratic in the carrier frequency from 25 kHz to 1 MHz. There is a seemingly faster decline above 1 MHz and the offset drops into background noise in the 2–5 MHz range.

The time constant of the offset displayed no obvious variation with either voltage or frequency.

Effects of Temperature

Figure 6*A* shows the effect of temperature changes on the normalized offset of nonelectrogenic cells; in this instance, the raw offsets have been normalized against that at 25 °C for each cell. The offset is seen to be monotone increasing in temperature from 5 to 35 °C. Figure 6*B* shows the effect of temperature changes on the normalized offset of electrogenic cells. The offset in this case is seen to be relatively independent of temperature. In both instances, temperatures of 40 °C or higher seemed deleterious to the cell; this is in accordance with previously reported observations (e.g., Pickard, 1974).

The conductivities of the electrogenic cells at different temperatures were measured by means of injecting square pulses of current through the signal pipette and across the cell membrane. The values observed for *Chara braunii* ranged from $0.6 \pm 0.2(6)$ S/m^2 at 10 °C to $1.5 \pm 0.3(6)$ S/m^2 at 30 °C, being $1.3 \pm 0.2(6)$ S/m^2 at 25 °C; for *Nitella flexilis* they ranged

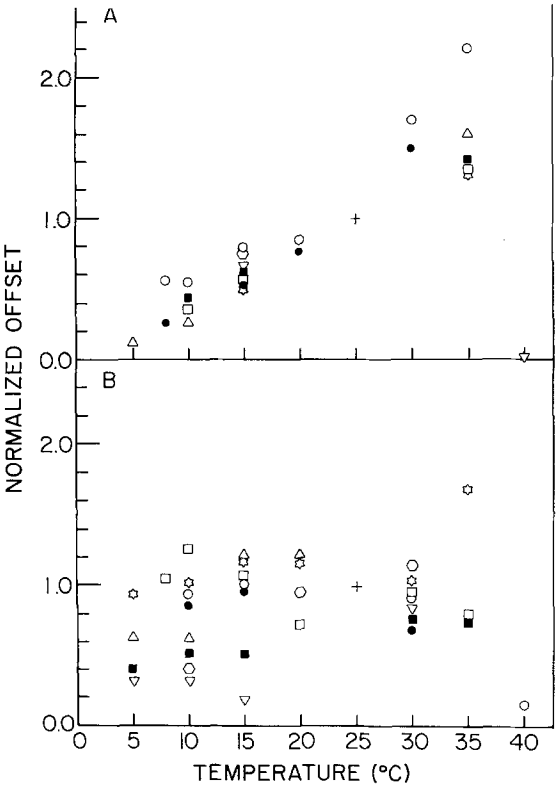

Fig. 6. Intracellularly observed offset at 4 V rms and 250 kHz for a number of Characean cells versus temperature of bathing solution; for each cell the offsets have been normalized to that at 25 °C. The hollow symbols are for *Chara braunii* and the solid symbols are for *Nitella flexilis;* the cross (+) denotes the normalization point. *A.* Nonelectrogenic cells. *B.* Electrogenic cells

from $0.7 \pm 0.2(5)$ S/m^2 at 10 °C to $1.7 \pm 0.4(5)$ S/m^2 at 30 °C, being $1.4 \pm 0.3(5)$ S/m^2 at 25 °C.

Effects of Metabolites

Since characean cells are known to take up sugars (*cf.* Wallen, 1974 and references therein), certain amino acids (Wallen, 1973), and amines (e.g., Walker, Smith & Beilby, 1979), and since these uptakes might be presumed to be active, it is not implausible that they might also involve electrogenic hydrogen ion antiport (or, equivalently, hydroxyl symport). To examine this possibility the bathing medium was modified to contain 5 mM glucose. In the four experiments performed neither the resting potential nor the offset revealed any clearcut change in either electrogenic or nonelectrogenic cells.

Effects of Fusicoccin

Fusicoccin, which is believed to influence a number of physiological processes among which is the stimulation of the electrogenic hydrogen ion extrusion system of plant cells (Marre, 1979) was added to the bathing solution in a 10 μM concentration. The measurements obtained from three *Chara* cells and one *Nitella* cell at 4 V rms, 250 kHz, and 25 °C were as follows: Cell 1 – nonelectrogenic *Chara braunii*: without fusicoccin, RP = −79 mV and $\tilde{V} = -750$ μV; after 20 min in fusicoccin-EAPW, RP = −112 mV (clearly out of the normal nonelectrogenic range) and offset = +312 μV. Cell 2 – nonelectrogenic *Nitella flexilis*: without fusicoccin, RP = −64 mV and $\tilde{V} = -1216$ μV; with fusicoccin, RP = −87 mV and offset = −1254 μV. Cell 3 – electrogenic *Chara braunii*: without fusicoccin, RP = −149 mV and $\tilde{V} = +237$ μV; with fusicoccin, RP = −182 mV and offset = +231 μV. Cell 4 – nonelectrogenic *Chara braunii*: without fusicoccin, RP = −53 mV and $\tilde{V} = -850$ μV; with fusicoccin, RP = −91 mV and offset = −775 μV.

It is well known that fusicoccin produces a hyperpolarization of the membrane potential (Marrè, 1979) and indeed this toxin produced a 20–40 mV hyperpolarization in both *Chara* and *Nitella* but did *not*, in the four experiments performed, significantly alter the radio-frequency offset unless it pushed the resting potential of the challenged cell out of the nonelectrogenic region and thereby flipped the membrane state.

Discussion

That the passive conductance mechanism might be different in an electrogenic state from that in a nonelectrogenic state is not a surprising conclusion since the states themselves are known to differ in various other essential aspects (*cf.* Walker, 1980). In the nonelectrogenic state, for example, the resting potential is potassium sensitive and putatively set by Nernstian mechanisms, whereas in the electrogenic state the resting potential is relatively insensitive to external cations and anions (Pickard, 1973), is quite sensitive to external pH (Roa & Pickard, 1976*b*), and seems due to electrogenic transport of H^+ (or OH^-). The fact, however, that this difference in charge transfer mechanisms is so trivially apparent using the radio-frequency irradiation technique is a startling result (*cf.* Figs. 2, 5, and 6).

When radio-frequency rectification in the Characeae was first proposed as an experimental tool (Pickard & Rosenbaum, 1978) it was shown that the rectification offset was expected to be an even function of the radio-frequency voltage applied across the cell membrane or approximately

$$\Delta V \cong \alpha_2 V_{rms}^2, \tag{1}$$

where ΔV [V] is the offset, α_2 [V^{-1}] is a proportionality constant characteristic of the membrane, and V_{rms} [V] is the root mean-square value of the drive voltage applied to the conductors of the microstrip exposure

apparatus. It seems therefore (*cf.* Fig. 2) that the mechanisms which underlie α_2 combine to produce a proportionality constant which is resting potential sensitive in the electrogenic state but not in the nonelectrogenic one. The key to understanding either type of variation of α_2 with the membrane electric field presumably lies in understanding the natures of the several classes of ionophores which are responsible for the translocation of ions across the cell's membranes; and understanding of this sort is beyond the present capabilities of plant electrophysiologists (*cf.* Pickard & Galanis, 1981).

The spontaneous flipping of offset during an experiment while the cell maintained a strongly negative resting potential ($\lesssim -160$ mV) suggests the possible existence of an as yet undescribed "hyperelectrogenic" cell state characterized by (*i*) strong electrogenic pumping and (*ii*) translocation mechanisms sufficiently finely balanced to give an α_2 which wanders between positive (*de*polarizing) and negative (*hyper*polarizing) levels. It could of course be argued that these observations were in some way artifactual, but this seems unlikely since: (*i*) no failure of the electronics was ever observed near the times that hyperelectrogenic observations were made and hyperelectrogenic cells tended to be interspersed with nonelectrogenic ones; and (*ii*) six of the seven hyperelectrogenic cells thus far encountered were grouped in a single three-week period which is in conformity with the observation that the state of our *Chara* culture drifts with time, some periods being characterized by mostly electrogenic cells and others by mostly nonelectrogenic ones. If the hyperelectrogenic state is real, it brings to four the number of discrete states identified for Characean cells, the fourth being that discovered by Bisson and Walker (1980) in which cells held at extremely high pH become, in effect, pH electrodes.

The theory of the rectification mechanism as developed to explain the offset in nonelectrogenic cells (Pickard & Barsoum, 1981), predicted that: (*i*) the offsets should vary as V_{rms}^2; (*ii*) as a result of membrane capacitance effects, the offsets should vary as f^{-2} in the middle radio-frequencies; (*iii*) the offset should display a transit time cutoff at sufficiently high frequencies. These predictions are seen to work for electrogenic cells (Figs. 4 and 5) as well as nonelectrogenic cells. However, for electrogenic cells the offset displayed a cutoff frequency of roughly 5 MHz, whereas for nonelectrogenic cells the cutoff was near 10 MHz (*cf.* Pickard & Barsoum, 1981).

If indeed the cutoffs observed in the low megahertz region are due to transit time effects, then the lower cutoff frequency (with higher resting potential) which characterizes the electrogenic state could imply significantly different intramembrane mobilities for the charge carrier(s) of the two states. For if $\hat{f}_c$ [Hz] is the cutoff frequency, L [m] the membrane thickness, and V_{RP} [V] the resting potential, then the intramembrane ionic mobility of the principal inward-going charge carrier can be estimated from (Pickard & Barsoum, 1981) $2\hat{f}L^2/|V_{RP}|$; and the inferred mobility in the electrogenic state is seen to be roughly one-fourth that in the nonelectrogenic state. These inferred mobilities most likely are associated with passive conduction processes since submicrosecond transit times seem a bit small for the number and nature of the steps commonly assumed to underlie an active translocation event. If this line of reasoning is valid, then the passive conduction mechanisms of the two states are grossly different as are the balances of ionophore behaviors which underlie the α_2's; and attempts to understand one state using conductance parameters measured in another are of distinctly questionable validity.

It seems that the variation of α_2 with temperature must also differ between states since the variation in the measured membrane resistivity with temperature did not. Thus, it seems as if in the nonelectrogenic state the mechanisms underlying α_2 combine to produce an $\alpha_2(T)/\alpha_2(25)$ which is monotone increasing whereas in the electrogenic state they combine to yield no discernible trend, a situation which suggests (*i*) that these mechanisms may be several in number and (*ii*) that they may buck one another in a complicated fashion.

The results for glucose, though not as extensive as those for frequency or voltage variations, revealed no clearcut change for either the resting potential or the offset. Therefore, either (*i*) the cells were not taking it up (we did no tracer studies concurrently) or (*ii*), given the measurements available, the sequelae of the uptake were too subtle to be resolved from the normal stochastic variations of resting potential and offset. Since the cells seemed otherwise robust, the latter possibility seems the more likely. However, since uptake may be expected to saturate at an external glucose concentration of about 10 mM (Wallen, 1974), the electrophysiological effects of the 5 mM glucose used here should be a fair fraction of those possible; therefore they must be presumed small, although just how small cannot be determined without additional data.

The results for fusicoccin showed no significant alteration in the radio-frequency offset in the absence of a change in electrogenic state. If it is assumed that our sparse data points are representative, one explanation could be that fusicoccin-induced H^+ extrusion is qualitatively different from normal electrogenic H^+ extrusion, a possibility which surely deserves more detailed investigation.

We believe that the results reported in this paper form the basis for broader in-depth studies of electrogenesis in plant cells in general and Characean cells in particular. Subsequent studies could involve, for instance, the utilization of a mixed voltage and current clamping technique by which a cell's vacuolar potential could be current clamped in the vicinity of a desired level without actually voltage clamping it there and destroying the utility of the radio-frequency rectification technique. Furthermore, the metabolite question deserves a more detailed examination using cells in which such substances as aspartic acid and methylamine are tried in addition to glucose. With good fortune, these, and additional studies, could in time shed some light on questions which are as yet unanswered, such as what sort of ion transport mechanism causes the radio-frequency rectification offset to change sign between electrogenic and nonelectrogenic states?

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