J. Membrane Biol. 85, 245–249 (1985)

The Journal of
Membrane Biology

Theory of Negative Capacitance in Membrane Impedance Measurements

Jack M. Ferrier, Jack Dainty, and Stephen M. Ross
MRC Group in Periodontal Physiology and Department of Botany, University of Toronto, Ontario, Canada M5S 1A8

Summary. Three possible explanations of the negative capacitance seen in the *Chara corallina* membrane impedance are critically examined. These explanations are based on: (1) voltage-dependent channel kinetics; (2) electro-osmosis; and (3) extracellular negative capacitance. It is shown that the first two can produce negative capacitance only with parameters which differ by several orders of magnitude from measured values. The last mechanism can produce a very large magnitude negative capacitance, in the appropriate frequency range. Possible experimental tests are discussed.

Key Words *Chara* · membrane impedance · negative capacitance

Introduction

Electrophysiological measurements have shown a large magnitude negative capacitance at low frequency in giant algal cells (Coster & Smith, 1977; Chilcott et al., 1983; Ross et al., 1985). The purpose of this paper is to examine theoretically mechanisms which may account for this effect. A negative value for the measured capacitance results when the applied voltage required for a given current decays with time, or when the applied voltage is found to lead the applied current at a given frequency. It can easily be shown that $-\omega RC = \tan(\theta)$, where ω is angular frequency, R is measured resistance, C is measured capacitance, and θ is the phase angle by which the voltage leads the current.

One mechanism which can result in a negative capacitance is that of ion channels with voltage-dependent kinetics. A population of such channels, in which the probability of opening and closing can be modified by changes in transmembrane voltage, would relax toward a new total conductivity in response to a change in applied voltage. Depending on the voltage dependence of the kinetic parameters, the voltage would either lead or lag the current.

Another process that can affect measured capacitance is that involving electro-osmosis and the transport number effect (Fensom & Dainty, 1963; Barry & Hope, 1969; Smith, 1977; Barry & Diamond, 1984), in which ions will accumulate at one side and be depleted at the other side of the membrane. Depending on which of these (electro-osmosis or transport number effect) dominates, the component of diffusion potential across the membrane produced by these concentration changes will either lead or lag applied current.

A negative capacitance can also result from the following process in the extracellular medium (a similar process may also occur in the intracellular medium). Experimental work on giant algal cells has supported the existence of a proton or hydroxyl transport system that dominates membrane conductance (Kitasato, 1968; Spanswick, 1972; Saito & Senda, 1974; Lucas & Ferrier, 1980). If this is the case, injected current will be carried through the membrane largely by protons or hydroxyl ions. Away from the membrane, proton or hydroxyl transport will be carried almost entirely by diffusion (Ferrier & Lucas, 1979; Ferrier, 1981, 1983). At any point in the extracellular unstirred layer, the electrical potential gradient must drive a component of current equal to the difference between the total current and that part of the current carried by diffusion. For a sinusoidal current, the proton or hydroxyl diffusion-driven component of the current will lag the total current at every point in the extracellular unstirred layer. As a result, the current driven by the electrical potential gradient in the unstirred layer, and thus the potential gradient itself, will lead the current. The following discussion is in terms of a proton transport system, but the results will also apply quite well to a hydroxyl transport system.

Theory and Discussion

The opening and closing of channels is a stochastic process. The small signal admittance of a popula-

tion of voltage-dependent ion channels can be written as (DeFelice, 1981):

$$Y(\omega) = gN_o + g[V - E][(N - N_o)q' - N_o p']/[q + p + i\omega], \quad (1)$$

where g is the conductance of a single open channel, N_o is the number of open channels, N is the total number of channels, V is the transmembrane potential difference, E is the equilibrium potential for the channels, q is the frequency of channels changing from closed to open (in sec^{-1}), p is the frequency of channels changing from open to closed, and q' and p' are the derivatives of q and p with respect to transmembrane potential difference. The measured membrane capacitance would then be given by (Coster & Smith, 1977; Ross et al., 1985):

$$C(\omega) = Im[Y(\omega)]/i\omega = -qN_o[V - E][q'p/q - p']/[(q + p)^2 + \omega^2], \quad (2)$$

where we have also used $N = N_o(q + p)/q$.

Thus, the measured capacitance could be negative if the rate of opening of channels was sufficiently voltage dependent so that $q'p/q$ was greater than p'. However, the magnitude can only become larger as frequency decreases if ω^2 is greater than $(q + p)^2$. Measured lifetimes for channel closing are in the range 1 to 10 msec (Anderson & Stevens, 1973; Fishman, 1973; Sachs & Lecar, 1973; Conti et al., 1975; Ferrier et al., 1979; DeFelice, 1981; Sakmann & Neher, 1983). Thus, q should be at least 100 Hz, and $C(\omega)$ from Eq. (2) can have an increasing magnitude with decreasing frequency only if ω is much greater than 100 Hz. Since the measured negative capacity occurs only as frequency decreases below about 1 Hz, channel kinetics with measured lifetimes cannot account for this effect.

The other mechanisms to be examined are based on ion transport and concentration changes in the unstirred layers of the solution bathing the membrane. As will be shown, these processes inherently give rise to low frequency effects. When current is injected through a membrane, electro-osmosis may produce fluid flow across the membrane, which will produce ion concentration changes in the unstirred layer as ions are swept up or away by the fluid flow (Barry & Hope, 1969; Coster & Smith, 1977). Transport number discontinuities at the membrane/solution interfaces will also cause solute buildup or depletion in the unstirred layers. These time-dependent changes in ion concentration will result in a transmembrane voltage component which can either lead the current, if electro-osmosis dominates, or lag the current, if the transport number effect dominates.

In the other mechanism to be described, it is the potential gradient in the unstirred layer itself which leads the current. If there are differences in ionic mobility in the bathing solution, between those ion species which are mainly transported by the membrane and those which are not, this will give rise to a phase difference between the electrical potential gradient in the bathing solution and the current (Ferrier, 1981, 1983). These processes involving ion transport in the bathing solution will be given a unified treatment.

The transport of a particular ion species in the bathing solution can be described by the following equation:

$$J_i = -D_i \text{grad} C_i - [\sigma_i/(z_i F)]\text{grad} V + vC_i, \quad (3)$$

where J_i is the flux of species i, D_i is the diffusion coefficient, grad is the gradient operator, C_i is the concentration, σ_i is the electrical conductivity attributable to species i ($= z_i^2 F^2 C_i D_i/RT$), V is electrical potential, and v is fluid velocity.

An equation for electrical current density can be written as follows:

$$I = \Sigma_i z_i F J_i = -\Sigma_i z_i F D_i \text{grad} C_i - \sigma \text{grad} V, \quad (4)$$

where Σ_i indicates summation over all i, and $\sigma = \Sigma_i \sigma_i$. The electroneutrality condition, $\Sigma_i z_i C_i = 0$, has been used to eliminate the term with v.

Let us define I_d, the diffusion-driven current density, as

$$I_d = -\Sigma_i z_i F D_i \text{grad} C_i. \quad (5)$$

If the D_i were to be the same for all ion species, then I_d would be zero, by the electroneutrality condition. Using Eq. (5), and solving Eq. (4) for grad$V$ we have:

$$\text{grad} V = -(I - I_d)/\sigma. \quad (6)$$

Equation (3) can then be rewritten as:

$$J_i = -D_i \text{grad} C_i + (\sigma_i/z_i F)(I - I_d)/\sigma + vC_i. \quad (7)$$

If the main membrane-transported ion species have diffusion coefficients that are significantly greater than the average in the bathing medium (i.e., if they are protons or hydroxyl ions), then I_d can be comparable to I in regions where concentration gradients of those species have been established. Otherwise, I_d will be much less than I (Fer-

rier, 1981, 1983). The ion species with the greatest membrane conductance is the species that will most affect transmembrane potential. In the limiting case that one species carries all of the current through the membrane, we can write $I = z_t F J_t(x = 0)$, where t refers to the membrane-transported species. If it is also the case that σ_t is much less than σ, then we can rewrite Eq. (7) as follows:

$$J_t = -D_t \text{grad} C_t + v C_t. \tag{8}$$

The equation governing C_t can be obtained from Eq. (8) by using the continuity relation:

$$dC_t/dt = -\text{grad} J_t = D_t \text{grad}^2 C_t - v \text{grad} C_t. \tag{9}$$

It can be shown that an equation similar to Eq. (9) can be written for the total ion concentration.

Equation (9) can in principle be used to calculate the concentration change produced by the electro-osmotic fluid flow and by the transport number effect. However, this equation can be solved by analytical means only if the concentration change is small relative to the background value, so that the fluid-driven flux can be assumed to be constant. Then, we can write:

$$dC_t/dt = D_t \text{grad}^2 C_t. \tag{10}$$

The fluid flow-driven flux must be retained in the boundary condition at the membrane/solution interface:

$$D_t \text{grad} C_t = -(I/z_t F) + v C_t. \tag{11}$$

To be certain that Eq. (10) provides a good approximation to Eq. (9), we solved both Eqs. (9) and (10) using a numerical computer program, with Eq. (11) as the boundary condition at $x = 0$. The difference between the two calculations was found to be extremely small for any reasonable value of fluid flow.

The maximum change in concentration at $x = 0$, if electro-osmosis predominates, can be found from Eqs. (10) and (11) to be given by:

$$C_t(0) = C_t(L) - v C_t(L) L/D_t, \tag{12}$$

where $C_t(L)$ is the background level, and L is the width of the unstirred layer. The fluid velocity will be given by the product of the current and the electro-osmotic coefficient. For an upper value of the latter, we can use 10^{-8} m^3 coul^{-1} (Fensom & Dainty, 1963; Barry & Hope, 1969). This is equivalent to 50 moles of water moved across the membrane per mole of ions. For a current density of 10^{-2} A m^{-2}, this gives a fluid velocity of 10^{-10} m sec^{-1}. Then, using $D_t = 10^{-8}$ m^2 sec^{-1} and $L = 10^{-4}$ m, we have:

$$[C_t(0) - C_t(L)]/C_t(L) = -vL/D_t = -10^{-6}. \tag{13}$$

A relative change in ion concentration of this magnitude would have a negligible effect on membrane potential. We have carried through the time-dependent solution of Eq. (10), but shall not reproduce it here, since it only confirms what is evident from the result in Eq. (13).

If the first term on the right side of Eq. (11) is included in the solution, which is equivalent to including the transport number effect, v in Eq. (13) is replaced by $v - I/(z_t C_t F)$. This expression will be negative for any reasonable values of C_t and v, and the resulting capacitance should therefore always be positive rather than negative.

We now consider the last of the three possible mechanisms that can produce a negative capacitance. Under appropriate conditions, the transport of ions in the bathing solution can give rise to a large phase difference between the current and the electric potential gradient in the unstirred layer. If protons are the main membrane-transported species, then, as shown by Eq. (8), the transport of protons in the unstirred layer will be carried by diffusion, and possibly also by convection. However, the same values of the parameters used above to show that electro-osmosis will have a negligible effect on ion concentration also show that the convection term is negligible in Eq. (8).

We can see from Eq. (6) that, if I_d lags behind I at some angular frequency, ω, then the potential gradient will lead the current. The component of current carried by proton diffusion can be found by solving the diffusion equation, Eq. (10), for C_t, with $I_t = J_t z_t F = -D_t z_t F \text{grad} C_t$, and with the boundary condition: $I_t = I$ at $x = 0$. If $I = I_o \sin(\omega t)$, this gives:

$$I_t = I_o \exp(-kx) \sin(\omega t - kx), \tag{14}$$

where $k = (\omega/2D_t)^{1/2}$.

If we assume that the diffusion of all the other ion species can be described by an average diffusion coefficient, D_o, then it is easy to show, using the electroneutrality condition, that $I_d = (1 - b) I_t$, where $b = D_o/D_t$. Then, integrating Eq. (6), using I_t from Eq. (14), gives:

$$\begin{aligned}(\sigma/I_o L)\Delta V_u &= \sin(\omega t) \\ &+ [(1 - b)/(2^{1/2} kL)][(1 - \exp(-kL)\cos(kL))\sin(\omega t + 3\pi/4) \\ &+ \exp(-kL)\sin(kL)\sin(\omega t - 3\pi/4)],\end{aligned} \tag{15}$$

where ΔV_u is the difference in electrical potential across the unstirred layer. It can be seen that, since k is proportional to $\omega^{1/2}$, as ω becomes very large the right side of Eq. (15) becomes equal to $\sin(\omega t)$, and there is no phase shift between the electrical potential difference and the current.

The measured phase shift depends on the phase shift between the total applied potential difference and the current. It can be shown that the measured phase shift is given by:

$$\tan(\theta) = (\sin(\theta_m)\Delta V_m^o + \sin(\theta_u)\Delta V_u^o)/\Delta V^*, \quad (16)$$

where ΔV_m^o and ΔV_u^o are the amplitudes of the potential drops across the membrane and across the unstirred layer, and $\Delta V^* = \cos(\theta_m)\Delta V_m^o + \cos(\theta_u)\Delta V_u^o + \Delta V_e^o$, where ΔV_e^o is the amplitude of the potential drop across the bathing solution outside of the unstirred layer, while θ_m and θ_u are the phase shifts across the membrane and the unstirred layer. For most values of θ_m and θ_u, ΔV^* will be close to the total potential drop measured, ΔV^o. Since $-\omega RC = \tan(\theta)$, where R and C are the measured resistivity and capacitance (per unit area), and since a similar equation can be written for membrane capacitance, C_m, and θ_m will usually be small enough that $\tan(\theta_m) = \sin(\theta_m)$, we can rewrite Eq. (16):

$$C = [R_m C_m \Delta V_m^o - \sin(\theta_u)\Delta V_u^o/\omega]/R\Delta V^o. \quad (17)$$

From Eq. (15) we can obtain the following result:

$$\sin(\theta_u)\Delta V_u^o/\omega = BLI_o/(\sigma\omega), \quad (18)$$

where $B = [(1 - b)/2kL][1 - \exp(-kL)\sin(kL + \pi/4)]$. It can be shown by using series expansions that $B = (1 - b)(kL/2)$ for kL much less than 1. Since $k = (\omega/2D_l)^{1/2}$, we see that for sufficiently small ω:

$$\sin(\theta_u)\Delta V_u^o/\omega = (1 - b)L^2 I_o/[2\sigma(2\omega D_l)^{1/2}], \quad (19)$$

which will increase without limit as ω decreases. However, numerical calculations using a computer show that B becomes approximately constant at higher frequencies. If we assume that R is close to R_m, that ΔV^o is close to ΔV_m^o, and using $\Delta V_m^o = I_o R_m$, we can rewrite Eq. (17) as:

$$C = C_m - H/f, \quad (20)$$

where $H = BL/(2\pi R_m^2\sigma)$, and f is frequency.

The unstirred layer thickness, L, should be about 10^{-4} m. In the range of kL values we are interested in, from $kL = 0.4$ to 4.0 (for frequencies from 0.035 to 5 Hz), B is constant within a factor of 2, with a minimum value of 0.11. We have $\sigma = 3 \times 10^{-2}$ S m^{-1}, and measured values of R_m are generally about 0.7 S^{-1} m^2 (Coster & Smith, 1977). Using $B = 0.11$ then gives $H = 1.1 \times 10^{-4}$ farad m^{-2} Hz. As we can see from Eq. (20), C cannot become negative unless H/f becomes larger than C_m, which is about 10^{-2} farad m^{-2}. This will only happen with the calculated H value for frequencies less than 0.01 Hz. However, recent work by Smith and Walker (1983) indicates that the alkaline band resistivity is much less than for the cell as a whole, with minimum values of 0.12 S^{-1} m^2. Ogata et al. (1983) report qualitatively similar results using the water-film technique. This could mean that there are high conductance proton (or hydroxyl) pumps or channels only in the alkaline bands. Since most of the injected current would go through these high conductance regions, it is appropriate to calculate H using the membrane resistivity for these regions. Using $R_m = 0.12$ S^{-1} m^2, and also using $B = 0.21$ (which is valid for f close to 1 Hz), $H = 8 \times 10^{-3}$ farad m^{-2} Hz. Thus, measured capacitance would go negative for frequencies less than 0.8 Hz. This is very close to the frequencies at which the experimentally measured capacitances do go negative (Coster & Smith, 1977; Ross et al., 1985). According to this result, the frequency at which capacitance becomes negative is quite dependent on the resistivity of the high conductance alkaline bands. Thus, cells with a high conductance in the alkaline band would show negative capacitance at higher frequencies than cells with lower alkaline band conductance. This provides a possible explanation for the finding of Coster and Smith (1977) that not all cells exhibited negative capacitance. Furthermore, Chilcott et al. (1983), using the water-film technique, show that negative capacitance occurs more often in the alkaline band than in the acid band, which agrees with the predictions made here. The finding of Beilby and Beilby (1983) that negative capacitance occurs at 5 Hz during the brief period when R_m becomes extremely low during punch-through may also fit in with these predictions.

Of the three mechanisms examined in this paper, the first, based on voltage-dependent channel kinetics, cannot account for the measured negative capacitance using measured values for ion channel lifetimes. However, if there is a voltage-dependent control mechanism for membrane conductance with time constants of the order of 10 seconds or greater, then it could be possible to explain the negative capacitance on this basis. The possibility of a conductance control mechanism with such long time constants is supported by the existence of low-frequency membrane potential fluctuations (in the

range of 0.03 to 0.3 Hz), which have been measured in algal cells (Roa & Pickard, 1977; Ferrier et al., 1979), and in animal cells (e.g., Ferrier et al., 1982). Further experimental studies on the possible existence of a low-frequency conductance control system, and its voltage dependence, could determine if this mechanism can explain the negative capacitance.

The second mechanism, based on electro-osmosis, could not account for the negative capacitance unless the electro-osmotic coefficient was orders of magnitude greater than measured values. This is in accord with the general finding that convection is much less important in extracellular ion transport than is diffusion (e.g., Ferrier, 1983).

The third mechanism considered, based on diffusion-driven transport of protons or hydroxyl ions in the unstirred layer, could account for the measured negative capacitance. An experimental study on the relation between resistivity in the alkaline band and the occurrence of negative capacitance, and on the relation between extracellular conductivity and negative capacitance, could provide a definitive test of this explanation.

References

Anderson, C.R., Stevens, C.F. 1973. Voltage clamp analysis of acetylcholine produced end-plate current fluctuations at frog neuromuscular junctions. *J. Physiol. (London)* **235**:655–691

Barry, P.H., Diamond, J.M. 1984. Effects of unstirred layers on membrane phenomena. *Physiol. Rev.* **64**:763–872

Barry, P.H., Hope, A.B. 1969. Electroosmosis in membranes; effects of unstirred layers and transport number. *Biophys. J.* **9**:700–728

Beilby, M.J., Beilby, B.N. 1983. Potential dependence of the admittance of *Chara* plasmalemma. *J. Membrane Biol.* **74**:229–245

Chilcott, T.C., Coster, H.G.L., Ogata, K., Smith, J.R. 1983. Spatial variation of the electrical properties of *Chara australis*. II. Membrane capacitance and conductance as a function of frequency. *Aust. J. Plant Physiol.* **10**:353–362

Conti, F., DeFelice, L.J., Wanke, E. 1975. Potassium and sodium ion current noise in the membrane of the squid giant axon. *J. Physiol. (London)* **248**:45–82

Coster, H.G.L., Smith, J.R. 1977. Low-frequency impedance of *Chara corallina:* Simultaneous measurements of the separate plasmalemma and tonoplast capacitance and conductance. *Aust. J. Plant Physiol.* **4**:667–674

DeFelice, L.J. 1981. Introduction to Membrane Noise. Plenum, New York

Fensom, D.S., Dainty, J. 1963. Electro-osmosis in *Nitella*. *Can. J. Bot.* **41**:685–691

Ferrier, J.M. 1981. Time-dependent extracellular ion transport. *J. Theor. Biol.* **92**:363–368

Ferrier, J. 1983. A mechanism for the regulation of ligament width based on the resonance frequency of ion concentration waves. *J. Theor. Biol.* **102**:477–486

Ferrier, J., Dixon, J., Illeman, A., Dillon, E., Smith, I. 1982. Low-frequency voltage noise in a mammalian bone cell clone. *J. Cell. Physiol.* **113**:267–272

Ferrier, J.M., Lucas, W.J. 1979. Plasmalemma transport of OH^- in *Chara corallina*. II. Further analysis of the transport system associated with OH^- efflux. *J. Exp. Bot.* **30**:705–718

Ferrier, J.M., Morvan, C., Lucas, W.J., Dainty, J. 1979. Plasmalemma voltage noise in *Chara corallina*. *Plant Physiol.* **63**:709–714

Fishman, H.M. 1973. Relaxation spectra of potassium noise from squid axon membranes. *Proc. Natl. Acad. Sci. USA* **70**:876–879

Kitasato, H. 1968. The influence of H^+ on the membrane potential and ion fluxes of *Nitella*. *J. Gen. Physiol.* **52**:60–87

Lucas, W.J., Ferrier, J.M. 1980. Plasmalemma transport of OH^- in *Chara corallina*. III. Further studies on transport substrate and directionality. *Plant Physiol.* **66**:46–50

Ogata, K., Chilcott, T.C., Coster, H.G.L. 1983. Spatial variation of the electrical properties of *Chara australis*. I. Electrical potentials and membrane conductance. *Aust. J. Plant Physiol.* **10**:339–351

Roa, R.L., Pickard, W.F. 1977. Further experiments on the low frequency excess noise in the vacuolar resting potential of *Chara braunii*. *J. Exp. Bot.* **28**:1–16

Ross, S., Ferrier, J., Dainty, J. 1985. Frequency-dependent membrane impedance in *Chara corallina* estimated by Fourier analysis. *J. Membrane Biol.* **85**:233–243

Sachs, F., Lecar, H. 1973. Acetylcholine noise in tissue culture muscle cells. *Nature, New Biol.* **246**:214–216

Saito, K., Senda, M. 1974. The electrogenic pump revealed by the external pH effect on the membrane potential of *Nitella*. Influence of external ions and electric current on the pH effect. *Plant Cell Physiol.* **15**:1007–1016

Sakmann, B., Neher, E. 1983. Single-Channel Recording. Plenum, New York

Smith, J.R. 1977. Electrical Characteristics of Biological Membranes in Different Environments. Ph.D. Thesis. University of New South Wales.

Smith, J.R., Walker, N.A. 1983. Membrane conductance of *Chara* measured in the acid and basic zones. *J. Membrane Biol.* **73**:193–202

Spanswick, R.M. 1972. Evidence for an electrogenic ion pump in *Nitella translucens*. I. The effects of pH, K^+, Na^+, light and temperature on the membrane potential and resistance. *Biochim. Biophys. Acta* **288**:73–89

Received 16 October 1984; revised 4 February 1985