Adiabatic Heating and Membrane Excitation†

M. H. Hawton and W. J. Keeler

Department of Physics
Lakehead University
Thunder Bay, Ontario, Canada P7B 5E1

Abstract. Excitable membranes can be induced to show an increase in conductivity such as that encountered in the "action potential." We suggest that this transient condition may be the result of heating of the sites through which the ions pass. The heat could be generated by an adiabatic phase transition of the membrane lipids. Equations derived on the basis of these ideas give good agreement with voltage clamped current measurements in algae and perfused squid axon.

Introduction

Excitability of the biological membrane is associated with a temporary increase in membrane conductance. A wide variety of plant and animal cells as well as artifical membranes are excitable. The duration of the excitation varies in length from milliseconds in nerve to seconds in plant cells. The current carrying ions involved are also variable. Cells may be stimulated by electrical depolarization of the cell membrane or by sudden changes of temperature, pressure or ionic environment.

The conductivity of pure phospholipid membranes is very low and does not become comparable to that of natural membranes until certain proteins are added. It is generally accepted that these proteins act as pores or organic carriers for the current carrying ions. In excitable membranes most ions probably pass through pores in the otherwise insulating phospholipid membrane.

There are two important factors that effect ionic conductivity, the energy barriers that the ions must surmount in order to pass through the membrane and their average velocity in the membrane. The hydration energy of the ion is an example of an activation energy or barrier and is typically a few electron volts. If dehydration is required for the ions to contribute to conduction, the activation energy factor would be very temperature-sensitive since this barrier is over one hundred times kT at room temperature. Abbot, Hill and Howarth[1] observed that heat is given off by nerve cells in the rising phase of the action potential and most of it is reabsorbed during the falling phase. The measured heat is small, but could reflect a significant local temperature increase in regions

†Work supported by a grant from the National Research Council of Canada.

of the membrane. Lehoczki, Hevesi and Bálint[2] observed that detergent micelles act as thermal insulators and allow a large interior temperature increase before equilibrium with the external heat bath occurs. Straub[3] presents a model of oxidative phosphorylation that considers the phospholipid membrane as an insulating layer and argues that thermal phonons in proteins will be reflected many times before escaping. Thus heat evolved in an exothermic reaction in the cell membrane might become trapped for sufficiently long times to raise the temperature of the pores. Possibly only a small amount of the total heat evolved escapes from the membrane before it is reabsorbed in a subsequent endothermic process.

A number of possible explanations of this heating and subsequent cooling have been discussed by Abbot and Howarth[4] although they tend to predict less heating than is observed. There is another possible heat source in the cell membrane these authors have not discussed. The liquid crystalline to crystalline phase transition of the phospholipids, which is essentially a freezing of the phospholipids, is highly exothermic. This transition has been studied by differential scanning calorimetry, ESR, NMR and X-rays in artificial systems. Chapman[5] found the transition to be very sensitive to the nature and condition of the polar head groups. In particular, substitution of $-N(CH_3)_3$ for $-NH_3$ at the positive free end of the head group raises the transition temperature by 25°C. Removal of water from the polar region has a similar effect. Thus the dipolar heads might "trigger" the phase transition of the hydrocarbon tails and the orientation of the heads could in turn be influenced by electric fields. Träuble[6] measured the rate of the phase transition and found it to vary from milliseconds to seconds depending on the concentration of cholesterol and Ca^{++}. This is the same time dependence observed electrically for excitation as mentioned earlier. Hamel and Zimmerman[7] considered the electric field perpendicular to the membrane as equivalent to raising the temperature by pE/k where p is the dipole moment per head, E is the local electric field and k is Boltzmann's constant. The phospholipid head has a dipole moment of approximately 25 Debye and thus a voltage change of 25 mV is equivalent to a temperature change of about 20°C.

Is it reasonable to assume that a liquid crystalline to crystalline phase transition could take place in the membrane of a living cell? The effects would be hard to observe if the frozen regions represented a small fraction of the total membrane surface. The freeze etch electron microscopy of Speth and Wunderlich[8] indicates that large molecules such as proteins are excluded from the freezing region of membranes. Scott[9] discusses recent experiments that point to precipitation of spin labelled lipid components in biological membranes. It would appear that any technique using molecular probes might fail to detect the phase transition.

While the question of whether or not excitation is linked to a phase transition of the lipids is difficult to resolve experimentally, it can be argued on theoretical grounds that it would occur. Phospholipids in natural membranes exist just above or in the phase transition region.[10] Hamel and Zimmerman's[7] calculations indicate that a typical membrane depolarization is equivalent to a reduction in temperature of the order of 20°C which is sufficient stimulus to induce some of the normally liquid phospholipids to freeze and liberate heat. In order to obtain estimates for the temperature increase, we will use measured results from artificial systems. A model will then be used to try to fit results for voltage clamped algal cells and squid axon.

Model

The physical mechanism of our model is the following:

(1) Depolarization of the cell membrane, pressure or change of ionic environment, acts as a driving force on the liquid crystalline to crystalline phase transition of some of the membrane lipids that are in a suitable local environment.

(2) The energy liberated by this exothermic phase transition raises the temperature of a fraction of the membrane surface. The amount of heat given off will vary with the magnitude and duration of the stimulus. This process takes milliseconds to seconds depending on the environment of the lipids.

(3) The membrane proteins are insulated by the lipids surrounding them and hence heat trapped in the pores will escape mainly through the interface between the pores and the solution. Here the current carrying ions exist in high concentration and have a high probability of absorbing the heat.

(4) This additional thermal energy available to the ions assists them in overcoming whatever barriers there are to entry into the pores — dehydration for example.

(5) The heat is gradually dissipated and the ion conductivity approaches a steady state.

(6) If the cell is not voltage clamped, the heat will be reabsorbed as the system returns to normal and the hydrocarbon chains melt. In unclamped cells the transition will generally not go to completion and hence the transient effects will be less pronounced.

We will now develop a mathematical relation which expresses the dependence of membrane current on time in terms of the assumption in our model. A detailed description of the phase transition is difficult and

will not be attempted at this time. The initial rate of heat liberation will be relatively large because the driving force is large and there is a maximum amount of liquid hydrocarbons. Thus, the heating can be described approximately by the function $Bexp\ (-\alpha t)$ where B is a measure of the total heat liberated and $1/\alpha$ is the time constant for the liberation process. The rate of temperature change at the pores will be the difference between this heating due to the lipid transition and losses to the environment which will be proportional to the temperature difference, ΔT, between the pores and the solution. Thus, in the pore

$$\frac{d(\Delta T)}{dt} = Bexp(-\alpha t) - D\Delta T \tag{1}$$

where D is a constant that determines the cooling rate. This equation is linear first order and can be integrated to give

$$\Delta T = \Delta T_0 [exp(-Dt) - exp(-\alpha t)] \tag{2}$$

where $\Delta T_0 = B/(D-\alpha)$ is the maximum temperature increase.

Membrane current is usually calculated using the one dimensional electrodiffusion equation and the constant field approximation.[11]

$$J_i = q_i(-\mu_i kT \frac{dC_i}{dx} + q_i E \mu_i C_i) \tag{3}$$

where i represents the ith ion species, C_i is its concentration, q_i its charge and μ_i its mobility. E is the field strength which is assumed independent of x, the distance from the outside of the membrane. J will be assumed to be independent of x as is usual. Equation (3) can then be integrated to give

$$J_i = \frac{q_i^2 \mu_i V/d\ [-C_{io} + C_{il}\ exp(-q_i V/kT)]}{1 - exp(-q_i V/kT)} \tag{4}$$

where d is the membrane thickness, and C_{il} and C_{io} are the concentrations of the ith ion on the inside and outside of the membrane, respectively. J_i is positive if the current is outward. The membrane voltage $V = -Ed$ is positive if the potential is lower inside. The total membrane current J is the sum of the contributions of the individual ions,

$$J = \sum_i J_i . \tag{5}$$

To decide which terms in Equation (4) show a significant temperature dependence, it is necessary to estimate ΔT_0, the maximum temperature increase. In scanning calorimetry experiments, Ladbrooke *et al.*[12] found that 2 to 12 calories/gram of heat were absorbed by the lipid 1,2-dipalmitoyl-L-lecithin during a crystal to liquid crystal transition. Transition temperatures T_c varied from -22°C (dioleoyl lecithin) to 40°C for different phospholipids and varying amounts of cholesterol and bound water. Steim[13] observed

that 3 calories/gram were absorbed by lipids extracted from cells of *Mycoplasma laidlawii*. Thus, cells in the liquid crystalline state (above T_c) may be stimulated to give up the heat of transition Q and T_c may vary over a wide range depending on the particular phospholipids in the cell membrane. If the phase transition is essentially adiabatic, temperature will increase to a maximum and then gradually decrease as heat is dissipated. For a specific heat C of ½ calorie/gram degree (a reasonable value for organic compounds) one obtains as an estimate of the peak local temperature increase $\Delta T_0 \leqslant Q/C \simeq 4$ to 24°C.

The dependence of J on temperature will be shown to be mainly due to the mobility since V is too small to make the $exp(-q_i V/kT)$ term change significantly. If T increases from T_0 to $T_0 + \Delta T$ for an energy barrier u, then

$$\begin{aligned} exp\,[(-u/k)/(T_0 + \Delta T)] &\approx exp\,[-(u/kT_0)(1 - \Delta T/T_0)] \\ &\approx exp\,(-u/kT_0)\ exp\,[(u/kT_0)(\Delta T/T_0)]\ . \end{aligned} \tag{6}$$

If $\Delta T < 25°C$, $u = eV$, $V = 100\ mV$ and $kT_0/e = 25\ mV$, then $exp(-eV/kT) < exp(-eV/kT_0) \times 1.2$. This is not large enough to account for the observed conductivity changes. The mobility μ_i includes the factor $exp(-\Delta\phi_i/kT)$ since the ions must overcome the energy barrier $\Delta\phi_i$,

$$\mu_i(T) = \bar{\mu}_i\ exp(-\Delta\phi_i/kT) \tag{7}$$

where $\bar{\mu}_i$ is a temperature independent factor. Using $\Delta\phi_i = 1\ eV$ (23 Kcal/mole) for potassium,[14] and assuming $\Delta T_0 = 15°C$ then, since $1/kT_0 = 40\ eV^{-1}$,

$$\begin{aligned} exp(-\Delta\phi_K/kT) &\approx exp(-\Delta\phi_K/kT_0)\ exp[(\Delta\phi_K/kT_0)(\Delta T/T_0)] \\ &= exp(-\Delta\phi_K/kT_0)\ exp(40 \times 15/300) = 7\ exp(-\Delta\phi_K/kT_0)\ . \end{aligned}$$

Here the mobility is increased by a factor of 7, a very significant effect. In the following derivation, only the effect of temperature on the mobilities will be considered.

From Equations (4) and (6)

$$\begin{aligned} J_i(T_0 + \Delta T) = \frac{q_i^2 V}{d} \frac{[-C_{io} + C_{il}\ exp(-q_i V/kT_0)]}{1 - exp(-q_i V/kT_0)}\ \bar{\mu}_i\ exp(-\Delta\phi_i/kT_0) \\ \times\ exp[(\Delta\phi_i/kT_0)\ (\Delta T/T_0)] \end{aligned}$$

i.e.,

$$J_i(T_0 + \Delta T) = J_i(T_0)\ exp[(\Delta\phi_i/kT_0)\ (\Delta T/T_0)]. \tag{8}$$

Since ΔT is given by Equation (2), the current will increase as heat is liberated by the lipids and then decrease as the heat is dissipated. The net result is a transient increase in current and conductivity. The temperature increase will favour the ion with the greatest $\Delta\phi$ and hence the main current

carrying ion may change. Thus, it is possible that the direction of the current can change due to this effect alone.

Comparison with Experimental Results

Algae. Our prediction will be compared directly with voltage clamped current *versus* time curves for Nitella translucens and Chara australis. It has been observed in radioactive tracer experiments that an efflux of chloride ions occurs when an algal cell is stimulated. Thus, there is good reason to assume that a chloride mobility increase is responsible for the negative current during excitation. Indeed, Gaffey and Mullins[15] measured a 20 fold increase in the chloride ion current in chara. As an estimate for the concentrations of ions in plant cells, we use the values given by Strunk:[16] $C_{KO} = .1$, $C_{NaO} = 1$, $C_{ClO} = 1.1$, $C_{KI} = 93$, $C_{NaI} = 37$ and $C_{ClI} = 130$ mM/litre.

Earlier, Hogg *et al.*[14] obtained activation energies $\Delta\phi_{Na} = .5\ eV$ and $\Delta\phi_K = 1\ eV$ and a permeability ratio $\mu_{Na}/\mu_K = .3$ at 291°K. Using this information plus the fact that chloride ion currents are small in the resting state, we must estimate the activation energy for a chloride ion and its mobility ratio with potassium. The net positive current is proportional to $C_{KI} + (\mu_{Na}/\mu_K)\, C_{NaI} + (\mu_{Cl}/\mu_K)\, C_{ClO}$. At all temperatures, this will be mainly a potassium current due to the small value of C_{ClO} and C_{NaI} and the small $\Delta\phi$ of sodium. Net negative current is proportional to $C_{KO} + (\mu_{Na}/\mu_K)\, C_{NaO} + (\mu_{Cl}/\mu_K)\, C_{ClI}$ where the main contributor is again potassium at T_0, but becomes chloride ions during excitation. Values which satisfy these conditions are $\Delta T \simeq 15°C$, $\Delta\phi_{Cl} \simeq 1.5\ eV$ and $\mu_{Cl}/\mu_K \leqslant 0.001$ at T_0. A good approximation for the current thus becomes

$$J = -J_{Cl}(T_0)\exp[(\Delta\phi_{Cl}/kT_0)(\Delta T/T_0)] + J_K(T_0)\exp[(\Delta\phi_K/kT_0)(\Delta T/T_0)]. \quad (9)$$

This equation yields the curves plotted in Fig. 1 along with experimental results for Chara[17] and Nitella.[11] The steady state values of potassium and chloride ion currents were chosen so as to give the best fit. Agreement is good considering the simplicity of the mathematical treatment.

Squid Axon. We will first consider measurements on perfused axons where it is possible to have only one current carrying ion and hence reduce the number of parameters necessary to define the current versus time curve. Adelman[18] reviews measurements on perfused voltage clamped axons where sodium was the main ion contributing to the current and discusses the fact that the sodium current does not go to zero after excitation but remains at a relatively high "plateau." The experimental current

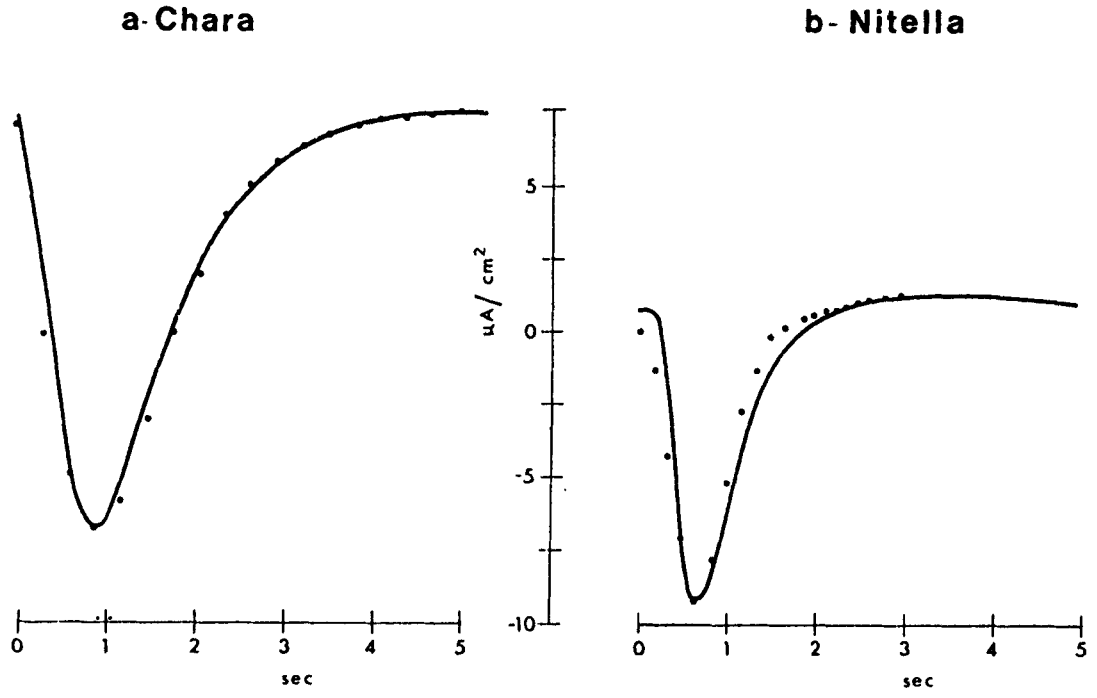

Fig. 1. Theoretical (solid lines) and experimental (points) current versus time curves during a voltage clamp. It is assumed that $\Delta T_0 = 15°C$.

(a) Chara australis (from experiments by Dainty, Lannoye and Tarr[17])

$\Delta\phi_{Cl} = 2eV$ and $\Delta\phi_k = 1eV$.

(b) Nitella translucens (from Cole[11])

$\Delta\phi_{Cl} = 3eV$ and $\Delta\phi_k = 2eV$.

versus time curves (see Fig. 2) show that the ratio of the peak to the steady state sodium current is approximately four which is of the same order as the change in algae. We will thus apply our model to perfused nerve and attempt to fit Adelman's curves in which potassium is removed from the system. The current as a function of time will be

$$J = J_{Na}(T_0)\, exp[(\Delta\phi/kT)\,(\Delta T/T_0)] \tag{10}$$

where

$$\Delta T = \Delta T_0\,[exp(-Dt) - exp(-\alpha t)]\ .$$

$J_{Na}(T_0)$, α and D were selected to give the best fit to the experimental results. The parameter α which determines the rise time was varied with clamp voltage since the heating rate depends on the free energy difference driving the transition.[19] Different steady state sodium conductances for ingoing and outgoing ions were used, that is, a different constant, $J_{Na}(T_0)$, was used for negative and positive currents. This gave a better agreement and is equivalent to assuming an asymmetric membrane, an assumption for which there is experimental evidence.[20] All other parameters are assigned for one curve and left unaltered for the rest. As can be seen from Fig. 2 there is a detailed agreement between experiment and our model for a large range of applied potentials. The voltage independence of all parameters except α is a severe test of the model.

We will next consider unperfused voltage clamped axons. Hodgkin and Huxley[21] in their analysis of their data find a factor of over 100 between the peak and steady state currents at some voltages. Our model does not predict these large ratios but rather it indicates that the sodium current follows a similar course to that in perfused potassium free axons. In spite of this, it gives reasonable agreement with currents in unperfused nerve at low membrane voltages where the potassium current is small. In Fig. 3 we compared the predictions of our model with the measurements of Narahashi.[22]

The activation energies for sodium and potassium conductance in squid axon can be estimated by consideration of the changes with temperature in the duration of the pulse and the total ion fluxes. Quantitative values can be expressed as a Q_{10} number where Q_{10} is the factor by which any quantity increases when the temperature is raised by 10°C. Guttman[23] reports that the duration of the conductance changes decreases with increasing temperature with a $Q_{10} = 1/3$. Landowne[24] showed that there is a net increase of sodium entry during activity with increasing temperature ($Q_{10} = 1.5$). On the other hand, the potassium loss per pulse decreases as the temperature is raised. These observations imply that the sodium conductance must increase sufficiently with temperature to compensate for the reduced pulse length. Since the average current during the pulse is the total sodium expelled divided by the duration of the pulse,

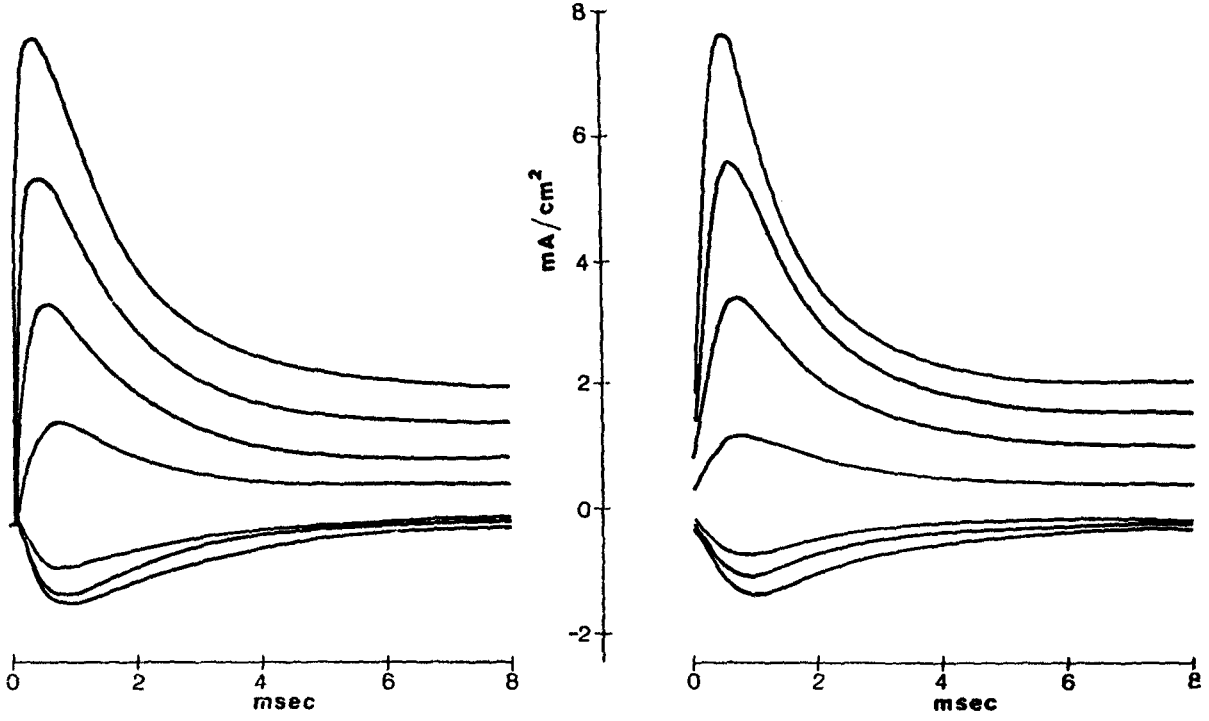

Fig. 2. **Left:** Membrane currents obtained in response to voltage clamping a squid giant perfused internally with 400 mM NaF and 130 mM CsF solution and externally with artificial sea water (taken from Adelman[18]). Potentials were -10, -5, 0, 20, 30, 40, and 50 mV. **Right:** Currents plotted according to equation 10 where i refers to Na. $\Delta\phi_{Na} = 1.1eV$.

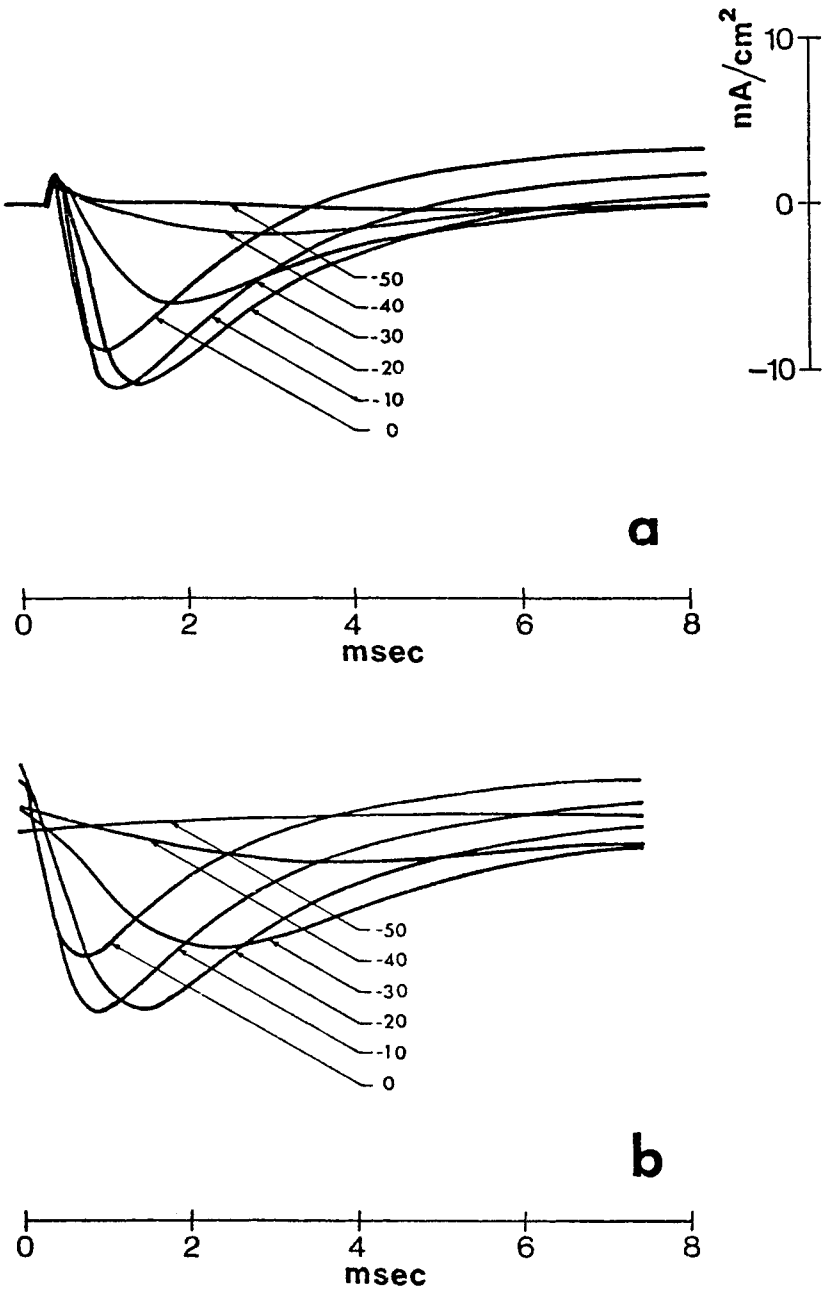

Fig. 3. a) Membrane currents obtained in response to voltage clamping an unperfused squid giant axon in artificial sea water (taken from Narahashi[22]). Membrane potential levels in mV during the voltage clamp pulses are indicated by arrows. b) Currents plotted according to equations 5 and 8 where potassium and sodium are the current carrying ions. $\Delta\phi_{Na} = 2eV$, $\Delta\phi_k \approx 0eV$.

Q_{10} = 1.5 ÷ 1/3 = 4.5 for sodium current changes. Using equation (6) where $T_0 \approx 300$ K and $exp[u/k(T_0 + 10)] = 4.5\ exp(u/kT_0)$ it follows that the activation energy, $u = 300/10 \times ln(4.5) \times 1/40\ eV = 1.13\ eV$. This is in agreement with the best fit value of 1.1 eV we obtained for perfused squid axon (Fig. 2). Since the value selected for ΔT_0, the maximum temperature increase due to the transition, is only an estimate, the very good agreement is somewhat fortuitous. Our assumption that the activation energy for potassium conductance is small is in agreement with the observed decrease in total potassium transferred as the temperature is raised (i.e. the pulse length decreases). The model we have presented appears to resolve "Landowne's paradox."

Conclusions

We have presented a possible, though speculative, theory of temporary conductance increases in phospholipid membranes. Our proposal is that the pores are heated by an exothermic phase transition of the lipids. If the activation energy for conduction is high this will result in an increased current. Ion currents that do not involve these high activation energies should not show this peaked transient behaviour, although some changes will occur due to "freezing." Effects other than heating, for example, conformation changes of the proteins during voltage clamp have not been included. Thus, we do not get detailed agreement in unperfused squid axon where this effect may be large. Knowledge of conformation changes would be required if one wished to explain quantitatively the difference between the steady state conductance in the resting and voltage clamped states. Finally, it is likely that temperature increase is only a first approximation to the process. The essential feature of the model is the transfer of energy from the freezing lipids or some other source to the current carrying ions. This energy could be carried directly as phonons or vibrations in the protein lattice in a way very similar to the model proposed by Straub[3] for oxidative phosphorylation.

Our theory is consistent with the recent dipole model of negative differential steady-state resistance.[7,25,26,27,28,29] This theory was used to explain the shape of the *i-v* curves of Gilbert and Ehrenstein[30] and the effect of polyvalent cations on these curves.

The conclusions are independent of any particular theory of ion conduction and are possible for a number of membrane structures. Although, for convenience, we have assumed that the ion currents are carried by pores, the arguments would still be valid if the ions passed directly through regions of the phospholipids. All that is required is a significant liberation of heat and a large activation energy. We feel that this model could explain some of the features of excitability and that it

deserves further study. The dependence of the transition temperature on voltage for a pure phospholipid system would be of considerable interest.

References

[1] B. C. Abbot, A. V. Hill and J. V. Howarth, *Proc. R. Soc. B.*, 148 (1958) 149.
[2] Lehoczki, Hevesi, and Balint, *Z. Prikl. Spekt.*, 16 (1972) 97.
[3] K. D. Straub, *J. Theor. Biol.*, 44 (1974) 191.
[4] B. C. Abbot and J. V. Howarth, *Physiol. Rev.*, 53 (1973) 120.
[5] D. Chapman, in the *Proceedings of the International Summer School on the Physics of Biological Membranes.* (To be published.)
[6] H. Trauble, *Naturwissenschaften*, 58 (1971) 277.
[7] B. B. Hamel and I. Zimmerman, *Biophys. J.*, 10 (1970) 1029.
[8] V. Speth and F. Wunderlich, *Biochem. Biophys. Acta.*, 291 (1973) 621.
[9] H. L. Scott, *J. Theor. Biol.*, 45 (1974) 241.
[10] Nagle, *Proc. Nat. Acad. Sci. USA*, 70 (1973) 3443.
[11] K. S. Cole, *Membranes Ions and Impulses*, (University of California Press, Los Angeles, 1968), p. 184.
[12] B. D. Ladbrooke, R. M. Williams and D. Chapman, *Biochem. Biophys. Acta.*, 150 (1968) 640.
[13] J. M. Steim, *Proc. N.A.S.*, 63 (1969) 104.
[14] J. Hogg, R. M. Williams and R. J. Johnston, *Biochem. Biophys. Acta.*, 150 (1968) 333.
[15] C. T. Gaffey and L. J. Mullins, *J. Physiol.*, 144 (1958) 505.
[16] T. H. Strunk, *Science*, 163 (1970) 84.
[17] J. Dainty, R. J. Lannoye and S. E. Tarr, *J. Exptl. Bot.*, 21 (1970) 558.
[18] W. J. Adelman, in *Biophysics and Physiology of Excitable Membrane.* W. J. Adelman, editor. (Van Nostrand Reinhold Co., New York, 1971), p. 312.
[19] M. E. Fine, *Introduction to Phase Transformations in Condensed Systems*, (The MacMillan Co., New York, 1964).
[20] D. E. Goldman, in *Biophysics and Physiology of Excitable Membrane.* W. J. Adelman, editor. (Van Nostrand Reinhold Co., New York, 1971), p. 346.
[21] A. L. Hodgkin and A. F. Huxley, *J. Physiol.*, 117 (1952) 500.
[22] T. Narahashi, in *Biophysics and Physiology of Excitable Membrane.* W. J. Adelman, editor. (Van Nostrand Reinhold Co., New York, 1971), p. 436.
[23] R. Guttman, in *Biophysics and Physiology of Excitable Membrane.* W. J. Adelman, editor. (Van Nostrand Reinhold Co., New York, 1971), p. 320.
[24] D. Landowne, *Nature*, 242 (1973) 457, see also p. 431.
[25] R. A. Arndt and L. D. Roper, *Bull. Math. Biophys.*, 34 (1972) 305.
[26] T. C. Ward and J. D. Bond, *Biophys. J.*, 11 (1971) 465.
[27] S. P. Almeida, J. D. Bond and T. C. Ward, *Biophys. J.*, 11 (1971) 995.
[28] L. Y. Wei, *Biophys. J.*, 12 (1972) 1159.
[29] D. E. Goldman, *Biophys. J.*, 4 (1964) 167.
[30] D. L. Gilbert and G. Ehrenstein, *Biophys. J.*, 9 (1969) 447.