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The Possible Role of Dielectric Constant Variation and of Electro-osmosis in Excitable Natural and Artificial Membranes. An Extention of Teorell's "Membrane Oscillator"

A. M. Monnier

Université Pierre et Marie Curie, Laboratoire de Neurophysiologie Ontogénétique, 9, Quai Saint Bernard, 75005 Paris, France

ABSTRACT - Artificial phosphatidic bilayers show ionic conductance and excitability only if they are in contact with cyclic ion-carriers and specific substances (Excitability inducing Materials). However many lipidic substances form ion-conducting and excitable membranes without any specific additives. They display a 10-20 fold transient increase of conductance under a weak field. Such "responses" repeat with a frequency which increases with the field. They are not a "noise" phenomenon and are similar to axon responses. The latter result, according to HODGKIN and HUXLEY, from the opening and closing of ionic pores. An alternative mechanism can be proposed. The lipidic membranes have a low dielectric constant. Therefore ions in membranes are bound as ion-pairs, formed by the cations and anions present in the membranes. The anions are always present in small amount because of a slight hydrolysis of the lipids. The dissociation constant of the ion-pairs (and thus the membrane conductance), as calculated by KRAUSS and FUOSS, is reciprocal to the interionic distance in the ion-pairs and to the dielectric constant. As the Na^+ ions are smaller than the K^+ ions the Na^+ containing ion-pairs are less dissociated than the K^+ containing pairs. Thus in the resting membrane the conductance due to Na^+ can be calculated 20-30 times smaller than the conductance due to K^+. Excitation could result from a slight increase of the dielectric constant of the membrane. A small afflux of water in the membrane, through the electroosmotic action of the stimulating current would suffice. Equations, similar to those proposed by TEORELL, describe the process.
The notion of ion-pairs leads to other interesting phenomena. Ion pairs in a low dielectric constant medium, containing a coloured material can be photo-dissociated. The radiant energy absorbed by the coloured substance suffices to overcome the association energy and therefore increases the conductance.

INTRODUCTION - Cells are surrounded by a thin lipidic membrane essentially constituted by phospholipids. Artificial phospholipidic bilayer membranes have been first proposed by MUELLER and RUDIN (10). However these membranes

display ionic conductance only if they are in contact with cyclic cation-carriers which possess inside hydrophilic groups, that can retain hydrated cations, and outside lipophilic groups. Thus cations can penetrate into the lipidic membrane. Phospholipidic bilayers are excitable only if they are in contact with specific substances such as EIM (Excitability Inducing Material). However, since 1964 (MONNIER et al.)(8, 9), and other authors BOTRE (2), KOBATAKE (6), have shown that artificial membranes, a few microns thick, ion conducting and excitable,can be made with many diverse lipidic material without any specific additives, except sometimes a small addition of fatty acid. These lipidic materials are drying oils, mono glycerides, alkyds, dioleyl-phosphate and even a common place substance such as bitumen. All these membranes under a voltage of 100-500 millivolts, show a transient response analogous to that of the axon membrane, i.e. a 10-20 fold increase of conductance. These responses usually repeat with a frequency that increases with the field. Their magnitude and regularity (fig. 1) show that they are not a "noise" phenomenon. The above membranes display excitability when inserted between identical aqueous solutions. On the contrary natural membranes are inserted between a K^+ solution

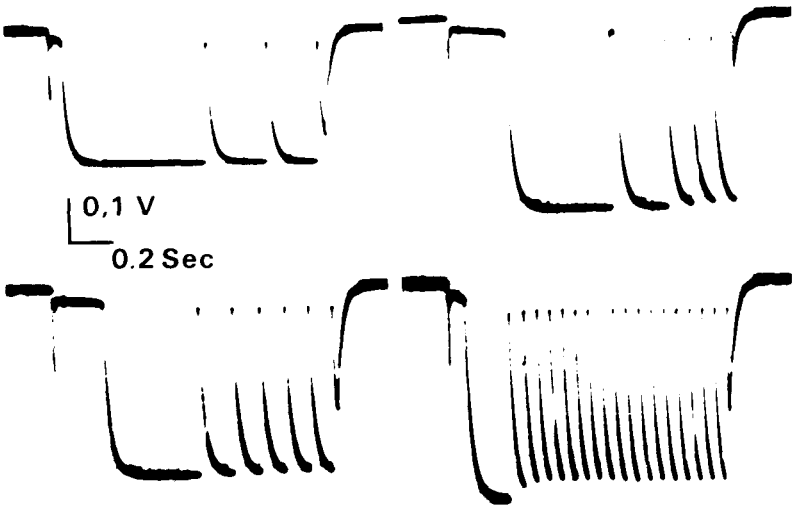

Figure I - Responses of a drying oil membrane under constant currents. The stronger the latter, the frequency of the responses increases and the latency diminishes (11).

and a Na^+ solution. However, at this present symposium TERAKAMA (15) has shown that the squid axon membrane can be excitable even when surrounded by identical solutions of the same cation. The results are best with either Co^{++} or Mn^{++} cations. It is to be noted that the cobalt cations and fatty acid anions, such as oleate, form salts that are not only soluble in water and alcohol but markedly soluble in oils and benzene. Thus they can be assumed to penetrate readily in lipidic membranes.

Therefore natural and artificial membranes can show excitability when surrounded by identical aqueous saline solutions. They can behave as monoionic systems. Of course one could apply to that case the classical theory of HODGKIN and HUXLEY : excitation can be attributed to the opening and closing of specific pores. But an alternative may be proposed. Membranes are structures of low dielectric constant D. In such systems fixed anions and mobile cations form ions-pairs. Conductivity of the membranes would depend upon the dissociation constant of these pairs, dissociation which is markedly dependent upon D. If the stimulating current increases D., excitation would result from an increased dissociation of ion-pairs.

INFLUENCE OF THE DIELECTRIC CONSTANT OF THE MEDIUM ON ION-PAIRING.

The dissociation of ion-pairs in medium of low dielectric constant D has been computed by BJERDUM (1) and by KRAUSS and FUOSS (7). This latter author (3) has proposed an approximate but easily manageable equation expressing the dissociation constant K_d of an ion pair

$$K_d = \frac{3000}{4\pi\, \mathring{a}^3\, 10^{-24}\, N}\; e^{-\frac{\varepsilon^2}{k\, 10^{-8}\, \mathring{a}\, DT}} \qquad (1)$$

N = Avogadro's number : $6.02\ 10^{23}$
ε = ionic charge : $4.8\ 10^{-10}$
k = BOLTZMANN constant : $1.38\ 10^{-16}$
$\mathring{a}$ = interionic distance in Angströms
D = dielectric constant
T : absolute temperature

The three latter parameters have a very large incidence upon K_d because they are included in the denominator of an exponential term. An approximation, which suffices to display interesting consequences, consists in considering only the exponential term.

$$K_d = e^{-\frac{580}{\mathring{a}\, D}} \quad \text{at } 25^{o}C. \qquad (2)$$

As the anions are more or less fixed to the membrane and that in this case their mobility is certainly low, the membrane conductance g is :

$$g = \sqrt{K_d} = e^{-\frac{290}{\mathring{a}\, D}} \qquad (3)$$

Thus conductance depends mainly upon D and the interionic distance $\mathring{a}$ in ion pairs.

Let us consider first the role of the interionic distance $\mathring{a}$. This distance is smaller for Na^+ containing than for the K^+ ion pairs, because the radius of Na^+ ions (0.95 Å) is smaller than that of the K^+ ions (1.33 Å), at least in the non hydrated state. If we assume that the ionic end of the membrane fatty anions has a radius of 2.5 Å, the above formula permits to calculate the ratio $\frac{g_K}{g_{Na}}$ of the membrane conductance pertaining to K^+ and to Na^+ ions (table I) for various values of D. This for the resting axon membrane which contains K^+ and Na^+ ions, if we suppose that their concentration is the same.

TABLE I

$\frac{g_{K^+}}{g_{Na^+}}$ in the resting axon membrane	D
28	2.5
16	3.0
10.8	3.5
8.04	4.0
6.4	4.5

This ratio falls because the Na^+ conductance increases more than the K^+ conductance as D rises. In spite of the uncertainties in the above calculation, the table indicates that the ratio $\frac{g_K}{g_{Na}}$ is of the same order as that observed in the resting membrane, i.e. 20.

Eq. (3) shows that membrane conductance depends markedly upon D. Therefore excitation could result of an increase of D. How can D be increased by the stimulating current ? The immediate hypothesis is that this current transports water in the membrane through electroosmosis. Lipidic materials do not dissolve in water. But water can be dissolved in such materials. Some, such as phospholipids, swell in water. But all lipids dissolve some water. For instance when oleic acid is agitated in water, and then centrifugated, it contains more than 2 p. 100 of water. Correlatively D of this acid is increased because D of water is so much larger than that of the acid (80 versus 2.5). Therefore electroosmosis of water, under the stimulating current, could be the factor of excitation. The following argument is derived from TEORELL's formulation (13, 14). We shall consider only membranes working under monoionic conditions, that is with identical solutions on both sides. For small increases of water in the membrane we can assume that D is a linear function of the water content W of the latter. Thus

$$g = CW \tag{4}$$

$$gE = i = CWE \tag{5}$$

C being a constant and E the observed voltage across the membrane.

The velocity of the electroosmotic transport of water $\frac{dw}{dt}$ is proportional to the current i and also the density of the anionic fixed charges, that is to the membrane conductance g

$$\frac{dw}{dt} = iw - a\,(w-w_o) \tag{6}$$

w_o being the water content in the outside layer adjacent to the membrane. The term $(w - w_o)$ is proportional to the inside pressure which limits the electroosmosis water afflux.
But the water w_o in the outside layer varies according to :

$$\frac{dw_o}{dt} = b\,(w_e - w_o) - iw \tag{7}$$

w_e being the water concentration inside the membrane in the absence of current. The electroosmotic depletion of water in the outside layer is equal to its increase in the membrane proper. The two above equations lead to a differential

equation of the second order :

$$\frac{d^2w}{dt^2} + [(a + b) - i]\frac{dw}{dt} + [ab + (a - b)i]\, w \square abw_e \qquad (8)$$

It is an equation of the form :

$$\frac{d^2w}{dt^2} + A\frac{dw}{dt} + Bw \qquad (9)$$

Its discussion is conveniently carried by the use of KENNELLY's (5) notation B_o that is "bluntness of resonance"

$$B_o = \frac{A}{2\sqrt{B}} \qquad (10)$$

In this case :

$$B_o = \frac{a + b - i}{2\sqrt{ab + (a - b)\, i}} \qquad (11)$$

Let, for the sake of simplicity, b = a

$$B_o = \frac{2a - i}{2a} = 1 - \frac{i}{2a} \qquad (12)$$

When i is small B_o remains close to 1 and w displays a single aperiodic increase. As the voltage across the membrane E is inversely related to W (Eq. 5) a single aperiodic decrease of E is observed at the onset of the current. If i approaches 2a, B_o approaches zero, w and E show a damped oscillatory pattern. When i : 2 a, B_o = 0 and w and E display sustained oscillations.
The calculation could be extended so as to include bi-ionic membrane systems, such as the axon membrane. In spite of the many assumptions involved in the above argument it seems that TEORELL's equations (13, 14) can be applied to the

excitable membrane itself, this if the variations of D under electroosmosis is considered. This approach is supported by a recent work of TASAKI (12) who reports a swelling of the axon membrane during excitation.

PHOTODISSOCIATION OF ION-PAIRS IN MEDIA OF LOW DIELECTRIC CONSTANT.

If one of the member of the ion-pair is coloured, that is light-absorbing ; illumination of the system shall increase its ionic conductance. For instance the oleate of a cationic dye, such as malachite green, dissolved in a medium of low dielectric constant (such as oleic acid or toluene) will show a marked conductance increase whan illuminated. In other words dissociation of a coloured ion-pairs can be elicited by light-absorption. This is made possible because the absorbed radiant energy surpasses the dissociation energy. For instance the maximum absorption of a malachite green ion-pair is, in the red, around 7000 Å. If the quantum efficiency is assumed to be unity the radiant energy absorbed by one mole of the coloured cation approaches 41000 Cal. $mole^{-1}$. The association energy can be calculated from eq. 3.

$$\log_{10} g = - \frac{12600}{\mathring{a}\ DT} \qquad (13)$$

Experiments show that $\log_{10} g$ follow the above equation. If it is combined with eq. 14 which a generally used in biochemistry, the association energy ΔF_o is obtained.

$$\Delta F_o = - 1360 \log_{10} K_d \quad \text{at } 25^{\circ}C. \qquad (14)$$

$$\Delta F_o = 1360 \frac{243}{\mathring{a}\ D} \text{ cal. mole}^{-1} \qquad (15)$$

For an interionic distance of $\mathring{a}$ = 4 Å and a dielectric constant D of 3, ΔF_o = 27600 cal. $mole^{-1}$. The above figure is not excessively large. This explains the recently observed photodissociation of ion-pairs (16, 17). Fig. 2

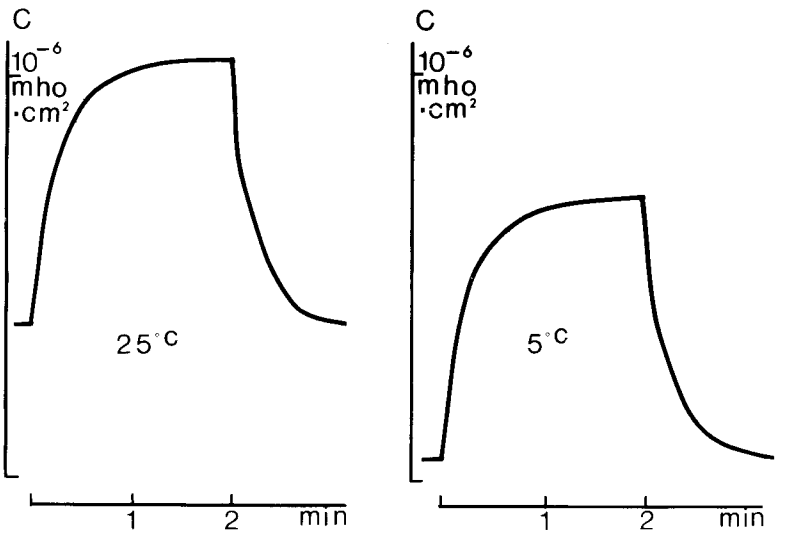

FIGURE 2 - Photoconductance of a drying oil membrane in contact with 0.1 MKCl solutions containing a chloride of a strong basic dye (malachite green, 1 g per 1000). Illumination (white light 2 x 10^4 lux) thermal effects avoided by adequate filter. Photoconductance is independent of temperature. But the dark conductance decreases markably with temperature.

Photodissociation of ion-pairs presents various aspects. Illumination does not always elicit a conductance increase of the system. If the coloured cation is a weak base, such as methylene blue, the photodissociation of the ion-pair methylene blue oleate liberates non-ionic species, that is oleic acid and the uncharged methylene blue molecule. Thus, in that case, illumination produced a smaller conductance.

For photodissociation to occur it is not necessary that the ion-pair contains a light-absorbing cation. A non-ionic dye such as SUDAN-III can be used. This dye is previously dissolved in oleic acid, which is then neutralized by KOH or NaOH. When this coloured alkaline soap is dissolved in a solvent of low dielectric constant, illumination produces a notable conductance increase. The oleate anion, when combined with the non-ionic dye, forms a light-dissociable ion-pair.

Photo-cells formed by ion-pairs. The naturally coloured copper ion permits the following experiment, showing the principle of a new photo-cell. A metallic copper electrode is placed at the bottom of a glass-container and covered by an aqueous solution of a copper salt ($CuCl_2$). This solution is covered by a layer of solvent of low dielectric constant (toluene) containing copper oleate. A copper electrode is immersed in the layer. Both electrodes are connected to an electrometer. The Cu^{++} concentration of the aqueous layer is adjusted

until the observed potential is zero. Then the activities of Cu^{++} ions in both layers are the same. But if the upper layer is illuminated the electrometer shows a potential difference which may attain 250 millivolts, under an illumination of 5000 lux (white ligth). Thus under this illumination the concentration of free Cu^{++} ions increases in an important measure. This reversible system behaves like a photocell. But its efficiency is, for the time being, very small.

CONCLUSION

The concept of ion-pairs in media of low dielectric constant deserves to be introduced in membranes biophysics. From this concept several features of natural and artificial membranes bearing upon their ionic conductance can be accounted for. This concept offers thus an anternative that can parallel the actual pore theory.

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Adress for reprint requests:

Alexander Monnier,
25 rue Gazan,
750 14 Paris,
France