BBA 75531

THE GENERATION OF "SPIKES" BY LIPO-COLLAGEN MEMBRANES

CLAUDIO BOTRÈ, WIM DORST, MARCELLO MARCHETTI, ADRIANA MEMOLI AND EMILE M. SCARPELLI

Istituto di Chimica Farmaceutica, Università di Roma, Rome (Italy)

(Received June 15th, 1970)

SUMMARY

Induced polarity in a collagen matrix by treatment with detergent provided a membrane system with an energy source suitable for "excitation" and thus capable of prolonged oscillation. Spontaneous generation of spike potentials of different amplitude and duration was observed in lipo-collagen membranes when a direct current field was applied.

The electrical oscillatory activity put a tremendous strain on the mechanical properties of the membrane caused by the peculiar properties of the protein matrix and its sensitivity to ionic strenght and species. A qualitative interpretation of the effect is presented on the basis of both Katchalsky's and Kirkwood's theories.

INTRODUCTION

Oscillatory membrane processes have been known for a long time and may be regarded as models for nerve impulse propagation.

TEORELL[1] and others[2] have studied oscillations in a single membrane by applying the combined polarity of osmotic gradient and electrical current.

SHASHOUA[3] modified this procedure by coupling two layers of opposite ionic permselectivity acting as perfect membrane electrodes.

MUELLER AND RUDIN[4] obtained a spike type oscillation in a bilayer system, similar to SHASHOUA's bipolar membrane, but containing a cyclic polypeptide as an ion carrier.

KATCHALSKY AND SPANGLER[5] have given a theoretical explanation for the spike effect based on non equilibrium thermodynamics and the molecular theory of polymer elasticity.

The peculiar mechano-chemical properties of collagen fibers and their sensitivity to ionic strength and species offered us an unique opportunity to verify if the coupling of electrochemical and structural properties of membranes can be considered responsible for spike potentials.

By choosing a surfactant molecule of suitable size and concentration so that permeation through the film was negligible, we succeeded in building a membrane

which we assume to be a bilayer of different nature and polarity. This under a direct current electric field can spontaneously generate spike potentials.

EXPERIMENTAL SECTION

Materials

Collagen films 25 μ thick were the generous gift of Ethicon Inc., Somerville, N.J. The preparation and properties of these membranes were described elsewhere[6]. Analytical grade NaCl, LiBr and HCl were used. Sodium lauryl sulphate was a sample supplied by British Drug House whose critical micelle concentration was found to be $7.9 \cdot 10^{-3}$ M by conductivity measurements at $25 \pm 0.02°$ in a thermostatic bath.

Procedure

The collagen film was placed between a two-compartment cell whose half-cells were filled by electrolyte solutions of equal concentration and identical composition, *i.e.* 0.1 M HCl or 0.1M NaCl or 0.1M LiBr. Then, one of the two compartments was added of 0.05 M sodium lauryl sulphate and the cell was allowed to stand for 24 h at room temperature to produce a bipolar membrane.

Leakage of the detergent from one half-cell to the other, was not observed either during conditioning or in the course of the following measurements. This was established by lack of any change in refractive index of the solution bathing the untreated side of the membrane.

After conditioning, the film, still clamped between the two cylindrical (perspex) half-cells, was washed several times and on both sides with the electrolyte solution to be used in the final measurements (0.1 M HCl or 0.1 M NaCl or 0.1 M LiBr). The apparatus was immersed in a water thermostat held at $20 \pm 0.2°$ and the solution in each half-cell was stirred constantly. When saline solutions were used, the pH was equal to 6.5. A digital pH meter Radiometer pH M52 was used for potentiometric measurements. In all cases saturated calomel electrodes and silver–silver halide electrodes were used indifferently.

For the direct current experiments, the treated membranes, clamped between two perspex half-cells, were separating two equal electrolyte solutions (0.1 M HCl or 0.1 M NaCl or 0.1 M LiBr).

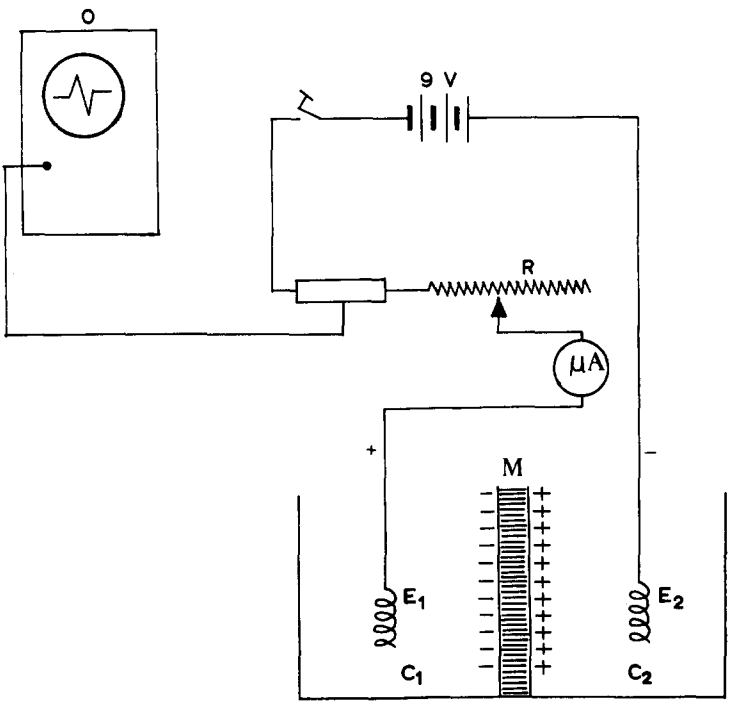
Silver–silver halide electrodes were used constantly. Any voltage change was amplified and displayed as voltage spikes on a Philips double beam P.M. 3230 oscilloscope. The firing patterns of a given membrane were photographed for further analysis.

The experimental arrangement is shown in Fig. 1.

RESULTS AND DISCUSSION

Potentiometric measurements (at zero electric current) carried out in different experimental conditions, using treated and untreated collagen films, indicate that conditioning with detergents changes the electrochemical behaviour of the protein matrix.

Results are given in Table I.



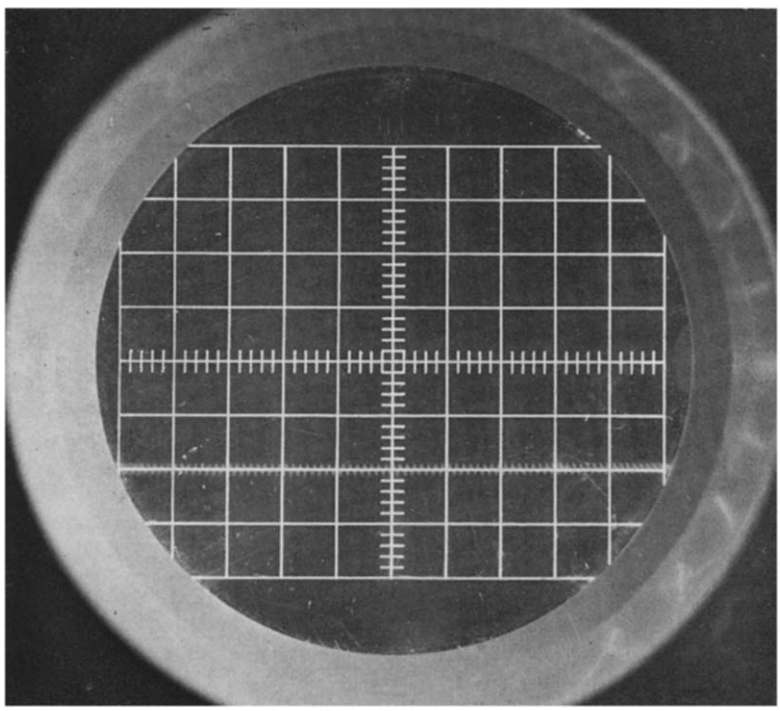


Fig. 1. Experimental arrangement: μA, microammeter; M, lipo-collagen membrane; O, oscilloscope; $C_1=C_2=0.1$ M halides; E_1 and E_2 are silver–silver halide electrodes.

TABLE I

Concn. ratio (M/M)	*Untreated membrane*		*Sodium lauryl sulphate treated membrane*	
	Electrical potential difference (mV)	*Transport number (t)*	*Electrical potential difference (mV)*	*Transport number (t)*
1/0.05 NaCl	18.2 satd. calomel electrode	0.6	2.5 satd. calomel electrode	0.5
0.1/0.05 NaCl	4.0 satd. calomel electrode	0.6	3.0 satd. calomel electrode	0.58
1/0.1 LiBr	24.0 satd. calomel electrode	0.7	14.0 satd. calomel electrode	0.6
1/0.5 HCl	32.0 Ag/AgCl electrode	0.9	24.0 Ag/AgCl electrode	0.7
1/0.1 HCl	102.0 Ag/AgCl electrode	0.9	87.2 Ag/AgCl electrode	0.7

The values of the electrical potential difference (in mV) given in Table I must be considered as extrapolations at zero time for typical runs (mV *vs.* time).

In the case of untreated membranes and when using saline solutions, the electrical potential data show that collagen possesses very weak permselectivity properties. In fact, in the experimental conditions given in Table I, the pH value (6.5) is within the isoelectrical "plateau" of the protein[7]. The theoretical values of the transport numbers for halide ions, calculated from the membrane potential data, are constantly higher than those found for the lipo-collagen membrane, indicating that after conditioning the membrane behaves as a bipolar system.

The same effect can be observed when pH values go far below the isoelectric point of the protein, as in the HCl experiments, and the protein matrix acquires a positive net charge which changes its permselectivity (see Table I).

If we assume that the change in the transport number for halide ions is proportional to the amount of negative charges introduced by the detergent, we may speculate that protein–detergent interaction is mainly of hydrophobic nature[8]. Otherwise, when conditioning a highly positive charged collagen film, as in the HCl experiments, the amount of detergent "complexed" to the protein matrix should be higher and consequently the change in transport number more significant. Following this assumption, we may estimate the negative charge density as equal to 0.02 equiv/kg of wet collagen film.

This falls within the stability range of coacervate systems[9]. The occurrence of such an arrangement between detergent and protein was found to be very critical and depending upon the time of conditioning. Usually, conditioning for less than 24 h produces membranes with a lower degree of stability and less capacity for oscillatory activity.

No significant difference was observed when the detergent concentration was above or below the critical micelle concentration. The passage of direct current through lipo-collagen membranes, separating two equal electrolyte solutions leads to spontaneous oscillatory activity of the membrane, which gives rise to spikes of varying amplitude (10–200 mV) and duration.

The firing pattern of the membrane, when using NaCl or HCl, resembles very much the one obtained by SHASHOUA[3] with polymeric salt films. In fact, firing occurs only at a critical applied voltage, its frequency increases with increasing voltage and

then suddenly ceases completely. If deviation from the critical current exceeds 2 %, oscillations are no longer observed.

The critical current values (I_{crit}) for the oscillatory activity of the membrane are given in Table II.

TABLE II

Electrolyte solution (M)	*Critical current (mA/cm^2)*	*Period (sec)*
0.1 HCl	100	0.5
0.1 NaCl	52	0.2
0.1 LiBr	54	0.2

These values were found constant for a given collagen membrane, as long as the same procedure for conditioning was followed. The oscillations last for about 10 min, then there is a constant current flow through the membrane, which looses its properties for spontaneous generation of spikes. This loss is irreversible, because at closer examination the membrane appears to be broken through in many parts.

When LiBr is used, the firing pattern is completely different in shape and magnitude.

A typical spike obtained with LiBr is shown in Fig. 2. Analysis of the shape of this spike shows that the voltage raises very quickly and steeply followed by slow decay and reversal of the trend. This reversal is of the same amplitude of the main spike and follows the same pattern.

We may now attempt a qualitative interpretation of our results in terms of KATCHALSKY's theory[5].

The current flowing through the membrane produces an accumulation of electrolyte within the membrane itself. This affects the protein matrix structure[10–12] and the whole membrane along with it, causing a tremendous shrinkage of the membrane and producing breakdown regions, with sudden electrolyte loss.

At the beginning only few regions of the membrane are involved and the firing activity is regularly spaced in time. Later on, the oscillatory pattern increases its frequency till the whole membrane is involved. At this point, the contraction of collagen fibers is so drastic that the membrane, being tightly clamped, tears apart.

This effect can even be predicted on the basis of KIRKWOOD's theory[13] without taking into account the mechano-chemical properties of collagen fibers.

We then derive

$$\begin{aligned} \frac{\mathrm{d}(\delta c)}{\mathrm{d}t} &= w\delta c + t_i K \frac{\delta E}{F} \\ \frac{\mathrm{d}(\delta E)}{\mathrm{d}t} &= \delta c \left(KRT \frac{t_i}{cFZ} + \frac{t_i RTw}{cF} \right) + \delta E \left(\frac{K}{Z} + \frac{t_i^2 RTK}{cF^2} \right) \end{aligned} \qquad (1)$$

where c is the electrolyte concentration, t_i is the transport number of ion i, K is the membrane conductance, E is the electric potential, Z is the membrane capacitance, w

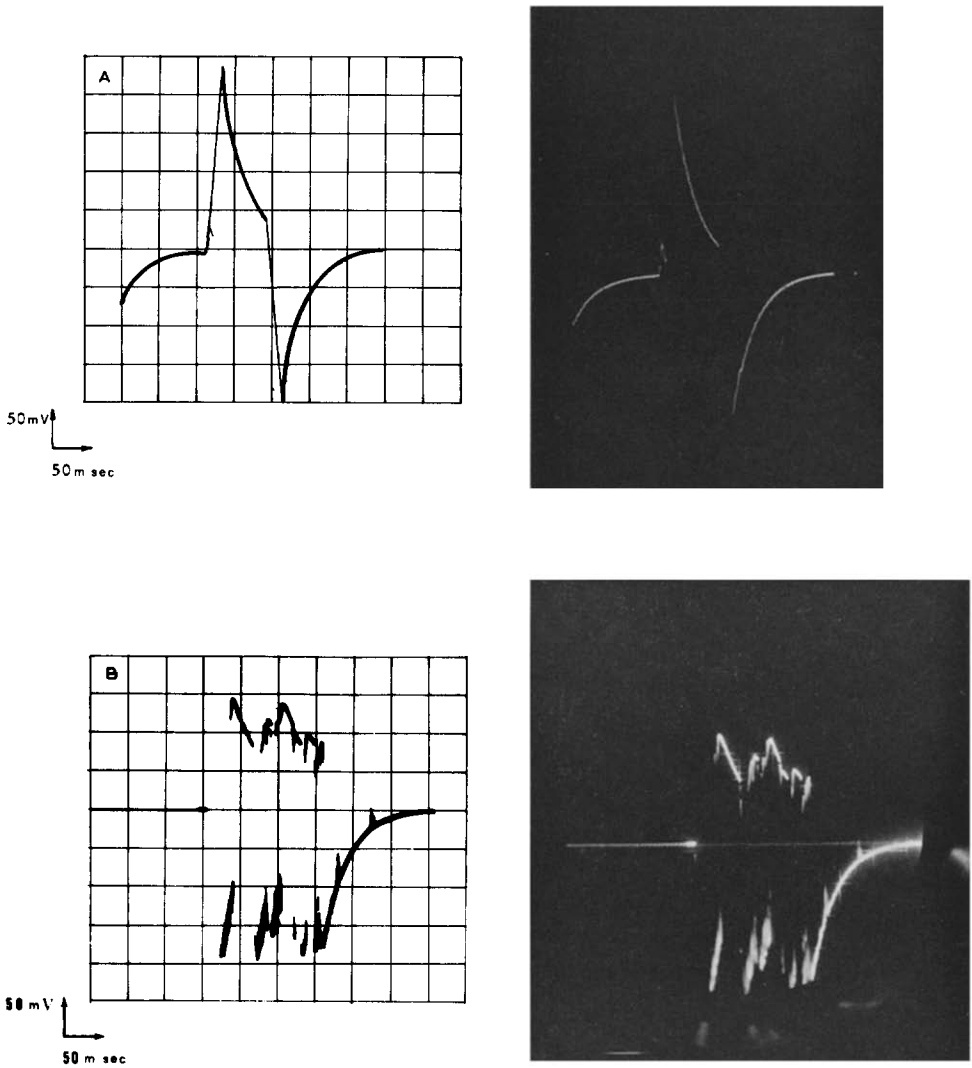


Fig. 2. Oscilloscope traces of spikes generated by lipo-collagen membranes when a direct current field is applied (A initial and B final stages).

is the osmotic electrolyte permeability, F is the Faraday, R is the gas constant and T is the absolute temperature.

The derivation of Eqn. 1 predicts electrolyte accumulation in the inner part of the membrane (between the two external layers which possess opposite charges and different charge densities). The same relation 1 predicts, qualitatively, permselectivity breakdown accompanied by a sudden electrolyte loss[14]. Yet Eqn. 1 will not show oscillations since the discriminant of the secular equation cannot become negative. However, a strong concentration dependence of t_1 must be taken into account.

We assume

$$K\delta(t_i E) = Kt_i\delta E - \frac{I\delta c}{c^2}$$
$$\delta(t_i c) = t_i\delta c - \frac{\delta c}{c} \qquad (2)$$

where I is the current density.

Inserting Eqn. 2 into Eqn. 1 we get the desired oscillations for a given ratio of transport quantities and external parameters.

The frequency of the oscillation is difficult to evaluate exactly in the present case. Nevertheless, the dependence on ionic species shown in Table II is elucidated by relations 2 and 1 since increasing the cofactor K will increase the critical current I.

Thus, induced polarity[15] provides a membrane system with an energy source suitable for "excitation" and gives prolonged oscillations as well. However, the phase transition in our synthetic lipo-protein system matches the external applied electrical field for a limited period.

A few years ago we observed[16] a sharp and sudden rise of voltage followed by a fast reversal, when titrating actin with myosin by a potentiometric method[17]. This was caused by a relevant change in counterion binding to the polymeric components when forming the actomyosin "complex". This effect took place so quickly that it gave rise to what can be considered a very rudimentary spike potential coupled to a physico-chemical reaction.

In living nature, enzymes provide a smoother intermediate between the driving mechanism (a chemical reaction) and the ionic currents.

REFERENCES

1 T. TEORELL, *J. Gen. Physiol.*, 42 (1958) 947.
2 K. R. PAGE, *Ph. D. Thesis, Aberdeen*, 1969.
3 V. E. SHASHOUA, *Nature*, 215 (1967) 846.
4 P. MUELLER AND D. O. RUDIN, *Nature*, 217 (1969) 713.
5 A. KATCHALSKY AND R. SPANGLER, *Quart. Rev. Biophys.*, 1 (1968) 127.
6 A. GLIOZZI, R. MORCHIO AND A. CIFERRI, *J. Phys. Chem.*, 79 (1969) 3063.
7 A. VEISS, *The Macromolecular Chemistry of Gelatin*, Academic Press, New York, 1964.
8 C. BOTRÈ, F. DE MARTIIS AND M. SOLINAS, *J. Phys. Chem.*, 68 (1964) 3624.
9 H. L. BOOIJ AND H. G. BUNGENBERG DE JONG, *Biocolloids and Their Interaction. Protoplasmatologia*, Bd 1/2, Springer, Wien, 1956.
10 L. V. RAJAGH, D. PUETT AND A. CIFERRI, *Biopolymers*, 3 (1965) 421.
11 D. PUETT, A. CIFERRI AND L. V. RAJAGH, *Biopolymers*, 3 (1965) 439.
12 A. CIFERRI, L. V. RAJAGH AND D. PUETT, *Biopolymers*, 3 (1965) 461.
13 J. G. KIRKWOOD, *Ion Transport Across Membranes*, Academic Press, New York, 1954, p. 119.
14 A. KATCHALSKY AND P. F. CURRAN, *Non Equilibrium Thermodynamics in Biophysics*, Harvard University Press, 1965, p. 173.
15 W. DORST AND C. BOTRÈ, *Il Farmaco Ed. Sci.*, 23 (1968) 399.
16 C. BOTRÈ, S. BORGHI AND M. MARCHETTI, *Biopolymers*, 4 (1966) 1046.
17 C. BOTRÈ, S. BORGHI AND M. MARCHETTI, *Biochim. Biophys. Acta*, 135 (1967) 208.