Biochimica et Biophysica Acta, 339 (1974) 367–373

BBA 76596

INTERACTION OF VASOPRESSIN WITH PHOSPHATIDYLSERINE BILAYERS

DIANA BACH and ISRAEL R. MILLER

Polymer Department, Weizmann Institute of Science, Rehovot (Israel)

(Received October 11th, 1973)

SUMMARY

Vasopressin causes a decrease in electrical resistance of phosphatidylserine bilayers. The magnitude of the decrease is a function of vasopressin and salt concentrations. The conducting channel is produced probably by aggregation of 4–5 molecules of the hormone.

INTRODUCTION

Vasopressin, a cyclic basic oligopeptide, causes an increase in water flow and sodium permeability in epithelia [1, 2].

Fettiplace et al. [3] reported that the hormone decreases the electrical resistance of lecithin–hexadecane bilayers at nearly neutral pH and it affects lecithin–decane bilayers at low pH values only.

Graziani and Livne [4] found a small increase in conductance of lecithin–decane bilayers after addition of vasopressin, but the initial resistance of their membranes was relatively low.

As we had previously studied interaction of basic polypeptides with different bilayers [5, 6] we decided to investigate the influence of vasopressin on the electrical resistance of phosphatidyl serine bilayers, having two aims in this research:

(a) to learn about the mechanism of the action of small basic polypeptides on the bilayers;

(b) to try to find a correlation between the action of vasopressin on biological and model membranes.

EXPERIMENTAL PROCEDURE

Vasopressin–lysine synthetic (Grade IV) was purchased from Sigma, St. Louis, Mo. Its biological activity was 90 international units/mg. The stock solution was prepared by dissolving the material in 10^{-3} M HCl (11 mg/ml).

Phosphatidylserine was obtained from Lipid Products (Nutfield Nurseries, Crab Hill Lane, South Nutfield, Great Britain) and the bilayers were formed from 0.5% solution in decane.

The aqueous phase was NaCl or KCl at different concentrations buffered to pH 7.1 by $5 \cdot 10^{-3}$ M Tris–HCl buffer. The temperature in all experiments was 36 °C. The technique of membranes formation and the electrical set up employed were as reported previously [5].

RESULTS AND DISCUSSION

Addition of vasopressin to the grounded stirred compartment after the film was black caused a drop in electrical resistance of the bilayers up to 2.5 orders of magnitude. The decrease is a function of the oligopeptide and salt concentrations.

Fig. 1 presents the current–voltage curve of the phosphatidylserine bilayer modified by addition of 3.3 μg/ml of vasopressin. The resistance of the modified bilayer is considerably lower than that of the intact phosphatidylserine bilayer which is higher than $3 \cdot 10^8$ Ω cm^2. The current across the unmodified bilayer at a potential of 100 mV only is about $4 \cdot 10^{-12}$ A which would make the i/V curve on the scale of Fig. 1 hardly discernible from the abscissa. The ohmic region of the modified bilayer is very narrow, above 30 mV the slope increases gradually, the dielectric breakdown is moved to $\approx$ 60 mV and it becomes even lower at higher vasopressin concentrations. The dielectric breakdown of the unmodified bilayer varied between 150 mV and 200mV and its resistance is ohmic up to 100 mV.

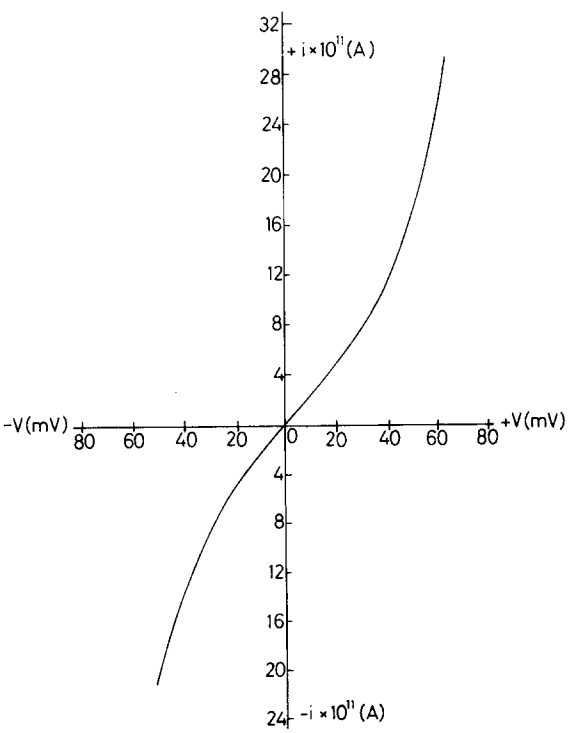


Fig. 1. Current–voltage characteristics of phosphatidylserine bilayer modified by addition of 3.3 μg/ml vasopressin. Aqueous phase, 10^{-1} M NaCl+5.10^{-3} M Tris–HCl, film area $1.1 \cdot 10^{-2}$ cm^2. Initial resistance of the film before addition of the oligopeptide $4.2 \cdot 10^8$ Ωcm^2.

Fig. 2 presents the final specific conductance of the bilayers as a function of the concentration of the added vasopressin. The time for reaching the final conductance values is very long; at the low concentrations of the oligopeptide the final values were obtained after about an hour from the time of the addition of the vasopressin and the membranes were quite stable at the low resistance. At the highest concentration employed (5.5 μg/ml), the time required to reach the low values was shorter, but the low resistance membranes only lived for a few minutes.

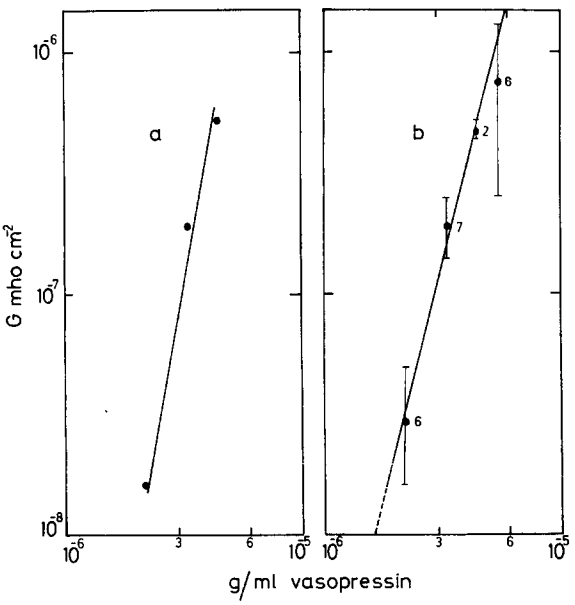


Fig. 2. Log of specific conductance of the bilayers as function of log vasopressin concentration. Aqueous phase, 10^{-1} M NaCl + 5.10^{-3} M Tris–HCl. (a) Subsequent addition on the same membrane after steady-state conductance values were reached. (b) Average conductance of several membranes; the bars indicate the range of the conductance for each concentration; the numbers represent the number of bilayers taken into account for each point.

Fig. 2a represents the steady state conductance (G) after subsequent addition of vasopressin on the same membrane.

Fig. 2b shows the conductance obtained by averaging the G of different membranes. In both cases the slope is between 4 and 5, indicating the possibility that the conducting channel is built by aggregation of 4–5 molecules of the vasopressin.

This structure formation by the vasopressin is in keeping with the destabilizing action of high concentrations of the oligopeptide on the bilayer.

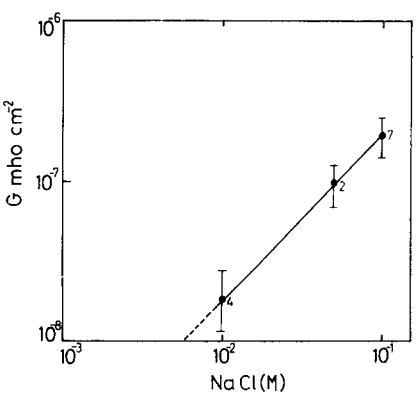
A molecular model of the vasopressin was built, from which it is possible to see that its length is $\approx$ 28 Å (extended side chain). This is too small to enable one molecule to traverse the bilayer; also the diameter of the ring is $\approx$ 2 Å, too small to permit the passage of hydrated cations (radius of hydrated K^+ is 2.17 Å and of hydrated Na^+ 2.28 Å [7]), so for traversing the bilayer vasopressin must aggregate.

No specificity with respect to K^+ or Na^+ was found as the same results (within experimental error) were obtained for bilayers modified by addition of 5.5 μg/ml

vasopressin in solutions of KCl or NaCl 10^{-1} M and 10^{-2} M or 10^{-1} M NaCl+ 10^{-2} M KCl.

It is difficult to decide what is the structure of the aggregates, but the pore formation is presumably completely independent on salt concentration as suggested by the slope 1 of log G vs log ([NaCl] (Fig. 3). This same dependence of conductance on salt concentration was obtained in phosphatidylserine bilayers modified by a copolymer lysine–phenylalanine [6]. It is also difficult at present to discern what the implications are of these findings on the action of vasopressin in biological membranes, especially as in biological membranes the hormone acts at much lower concentrations. On the other hand, Hays [2] proposed that the hormone influences water flow in toad bladder by increasing the number of pores, and this is in agreement with the data reported in this paper.

The deviation from the linearity of the current potential characteristic which occurs in the presence of vasopressin (Fig. 1) deserves more detailed consideration. The apparent increase in conductance with potential drop across the membrane is predicted by the barrier model theory of membrane conductance [8, 9], as well as electrostriction considerations. In Fig. 4 a schematic picture of the potential barrier to ion transport through the bilayer under the influence of an electrical field is presented. Considering only the transport in the direction of the field, namely, that of the ca-



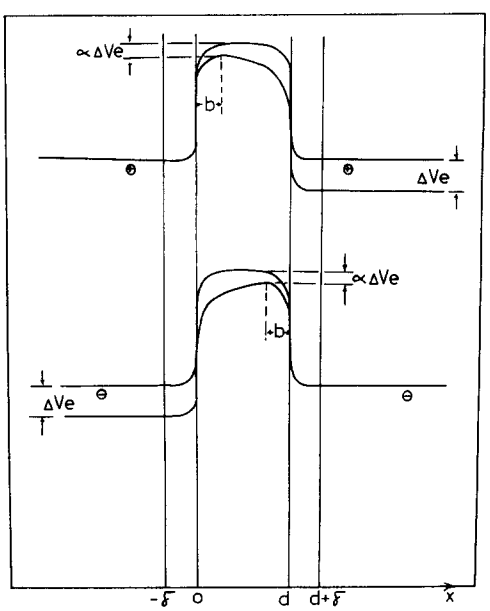


Fig. 3. Log of specific conductance of bilayers modified by addition of 3.3 μg/ml of vasopressin as a function of log NaCl concentration. In addition to NaCl the aqueous solution contained also 5.10^{-3} M Tris–HCl. Numbers and bars as in Fig. 2.

Fig. 4. Schematic representation of a potential barrier to transport of cations and anions across a bilayer membrane under the influence of an electrical field. d is the thickness of the hydrocarbon layer, δ the thickness of the polar layers, and b is the distance from the barrier peak to the nearest boundary of the hydrocarbon layer.

tions from the positive to the negative side, and of the anions in the opposite direction, and thus neglecting the back diffusion (this is justified at potentials above 20 mV), the barrier model renders the following expression for the dependence of current I on potential [8, 9]

$$I = A \exp \frac{\alpha \Delta V e}{kT} \left\{ C_1 k_+^0 \exp \frac{-\Delta G_0^+}{kT} + C_2 k_-^0 \exp \frac{-\Delta G_0^-}{kT} + B \Delta V \right\} \tag{1}$$

where A is the membrane area, ΔV is the potential drop across the membrane, e is the charge of an univalent ion, C_1 and C_2 are the salt concentrations on the two sides of the membrane, ΔG_0^+ and ΔG_0^- are the free energies of activation for the cation and anion transport in the absence of an electrical field, k_+^0 and k_-^0 are the diffusion-controlled transport rate constants and B is a quantity depending on the interaction of discrete charges across the membrane; α is the ratio of the distance of the potential peak from the hydrocarbon layer boundary b to the total width of the barrier d. It is analogous to the transfer coefficient in electrode reactions [10] but here it depends on the width of the membrane rather than on the symmetry of the barrier.

It is evident from Eqn 1 that $\exp(\alpha \Delta V e)/kT$ can be linearized for $(\alpha \Delta V e)/(kT) \ll 1$. The deviation from linearity will occur at higher potentials for lower values of α. However, as evident from Fig. 4 the value of α will increase with the thinning of the barrier. This thinning of barrier is likely to occur in the domains penetrated by the polypeptide molecules.

Electrostriction may also be responsible for the increase of conductance with the potential across the membrane. The electrostriction is exerted by the pressure of the polar molecules or groups dragged in the direction of increasing electrical field. For a rough estimate of this pressure, let us assume that the electrical field in the aqueous phase is zero and that it increases through the polar region of the bilayer until it reaches a constant value in the hydrocarbon layer (Fig. 4).

The electrostriction pressure p_e equals the total force acting on the Σn_i dipoles at the interface 1 cm^2 in area

$$p_e = \sum^{n_i} \bar{\mu}_i \frac{dE}{dx} \tag{2}$$

where $\bar{\mu}_i$ is the dipole moment of the ith dipole and (dE/dx) is the electrical field gradient acting on it. As a rough approximation let us replace Eqn 2 by

$$p_e = n \bar{\mu} E^0 / \delta \tag{3}$$

where n is the number of surface dipoles of the average dipole moment $\bar{\mu}$, E^0 is the electrical field in the hydrocarbon layer and δ is the thickness of the polar layer in which the electrical field is varying. The value of $n\bar{\mu}$ can be obtained from the measured surface potential ϕ_m of the respective monolayers: [11]

$$n\bar{\mu} = \frac{\phi_m}{4\pi} \tag{4}$$

As an example let us estimate the dependence of the electrostriction pressure on the potential ΔV across the bilayer with a polar region characterized by a surface potential $\phi_m = 0.75$ V. Let us assume the thickness of the hydrocarbon layer in the absence of an electrical field to be $d_0 = 50$ Å and let it be bounded by two 10 Å thick

hydrated layers of polar groups. Let us also assume, for the sake of simplicity, that the interfacial tension of the uncomplexed bilayer is close to zero and that of the hydrocarbon water interface γ is 50 dynes/cm. When the bilayer is compressed by electrostriction, a fraction θ of the hydrocarbon interface is revealed. As a first approximation the resulting surface tension γ equals $50 \times \theta$ dynes/cm. For an incompressible liquid hydrocarbon layer $\theta = \Delta d/d_0$, where Δd is the decrease in the thickness of the hydrocarbon layer. The resulting surface tension counteracts the thinning and expansion of the bilayer.

According to Eqns 3 and 4, the pressure exerted on the surfaces of the bilayer will then be

$$p_e = \frac{0.75 \times \Delta V}{12.5 \times 300 \times d \times 10^{-7} \times 300} = 6.7\ \Delta V/d \text{ dynes/cm}^2$$

where ΔV is given in volts. This pressure results in an expansion force of $6.7 \cdot \Delta V/d \cdot d = 6.7 \cdot \Delta V$ dyne acting on 1 cm of the hydrocarbon layer. This force is counteracted by the surface tension of the two bilayer interfaces which means that

$$2\gamma = 2 \cdot 50 \cdot \theta = 100 \frac{d_0 - d}{d_0} = 6.7\ \Delta V \tag{5}$$

or

$$d = d_0 \left(1 - \frac{6.7 \Delta V}{100}\right)$$

which means that a potential of 100 mV causes a thinning of the bilayer membrane only by less than 1% and an increase in surface tension by less than 0.5 dyne/cm. This is of course a low estimate of electrostriction, since there is an orienting interaction between the different surface dipoles resulting in a surface potential corresponding to apparently low surface dipole moments. It is therefore conceivable that the actual force of the inhomogeneous field acting on the surface dipoles may be larger by up to a few hundred percent. Thinning of the hydrocarbon layer in the domain of the penetrating polypeptide molecule results in an increase in E^0 and, therefore, according to Eqns 3–5 also in the relative electrostriction and on the height of the potential barrier to transport.

It follows from what has been said in this discussion that the lower potential of deviation from the ohmic resistance in the presence of vasopressin than in its absence can be explained by the dependence of the transfer coefficient α, as well as the electrostriction on the thickness of the hydrocarbon barrier.

ACKNOWLEDGMENT

This work was supported by NIH Grant No. 06-034-1.

REFERENCES

1 Fuhrman, F. A. and Ussing, H. H. (1951) J. Cell Comp. Physiol. 38, 109
2 Hays, R. N. (1972) in Current Topics in Membranes and Transport (Bonner, F. and Kleinzeller, A., eds), Vol. 3, p. 339, Academic Press, New York

3 Fettiplace, R., Haydon, D. A. and Knowles, C. D. (1972) J. Physiol. London 221, 18P
4 Graziani, Y. and Livne, A. (1973) Biochim. Biophys. Acta 291, 612
5 Bach, D. and Miller, I. R. (1973) J. Memb. Biol. 11, 237
6 Bach, D. (1973) J. Memb. Biol. 14, 157
7 Kavanau, J. Lee (1965) Structure and Function in Biological Membranes, Vol. 2, p. 327, Holden Day Inc., San Francisco
8 Miller, I. R., J. Memb. Biol., submitted for publication
9 Hall, J. E., Mead, C. A. and Szabo, G. (1973) J. Memb. Biol. 11, 75
10 Vetter, K. J. (1967) Electrochemical Kinetics, p. 134, Academic Press Inc., New York
11 Davies, J. T. and Rideal, E. K. (1963) Interfacial Phenomena, p. 71, Academic Press, New York