Biochimica et Biophysica Acta, 291 (1973) 612–620

BBA 76201

BILAYER LIPID MEMBRANE AS A MODEL FOR VASOPRESSIN, PROSTAGLANDIN AND Ca^{2+} EFFECTS ON WATER PERMEABILITY

YOSEF GRAZIANI and AVINOAM LIVNE

Negev Institute for Arid Zone Research and University of the Negev, Beer Sheva (Israel)

(Received July 31st, 1972)

SUMMARY

1. Bilayer lipid membranes were generated in an aqueous medium from egg phosphatidylcholine and the water permeability of the black membranes was determined by measuring the net volume flux produced by a NaCl gradient.

2. In agreement with previous reports, vasopressin increased the water permeability coefficient of the lipid membrane. Cyclic AMP, over a wide concentration range, had little or no effect on the water permeability, substantiating a direct effect of the antidiuretic hormone on the membrane.

3. Similar to observations in biological systems, Ca^{2+} and prostaglandin E_1 inhibited, apparently competitively, the action of vasopressin on the water flow of the model membrane. The inhibitory effects were observed if Ca^{2+} and prostaglandin E_1 were added prior to, but not after, vasopressin.

4. The formation of a vasopressin–lipid membrane complex stabilized by electrostatic and hydrophobic interactions is proposed.

5. Unlike valinomycin, neither vasopressin nor cyclic AMP changed significantly the electrical conductance of the bilayer membranes in a medium containing NaCl and KCl.

6. It is concluded that bilayer lipid membranes apparently possess the characteristics of the proposed site of action of vasopressin in biological membranes related to water transport, while the second site, related to Na^+ transport, is lacking.

INTRODUCTION

The stimulatory effect of vasopressin on water flow and Na^+ transport across biological membranes are commonly studied in frog skin[1], toad bladder[2], and renal systems of various rodents[3–5]. Vasopressin has been shown to increase the tissue level of cyclic AMP[6], and to stimulate adenylate cyclase activity[7], indicating that cyclic AMP is a mediator in vasopressin regulation of hydroosmotic flow in biological systems[8,9]. However, the role of cyclic AMP as a mediator for the vasopressin effect is controversial[10], and the mode of action of the proposed mediator is not clear.

The similarities between biological and artificial lipid membranes[11,12], particularly with respect to water permeability[13–16], raise the possibility of exploring the mechanism of vasopressin action in model membrane systems. Indeed, a direct effect

of vasopressin on water permeability of lipid bilayer membranes has been recently demonstrated[17,18]. A specific interaction between vasopressin and a lipid monolayer system has also been shown[19]. In terms of specificity and potency the effect of vasopressin on the water permeability of the bilayer membrane bears some intriguing similarities to the corresponding biological effects[17].

The marked interaction of vasopressin with prostaglandin E_1 (refs 20 and 21) and with Ca^{2+} (refs 22, 23) in affecting the permeability properties of biological systems, attracted us to examine these parameters in the model system. This communication records our results and illustrates the usefulness of the lipid model system for the study of the mode of action of vasopressin. An abstract of part of this report has appeared[24].

MATERIALS AND METHODS

Permeability measurements

The osmotic flux across bilayer phosphatidyl choline membranes was measured according to described procedures[15,25]. A diagram of the apparatus used is shown in Fig. 1. In principle, a thin-lipid membrane was formed between an open (100 ml)

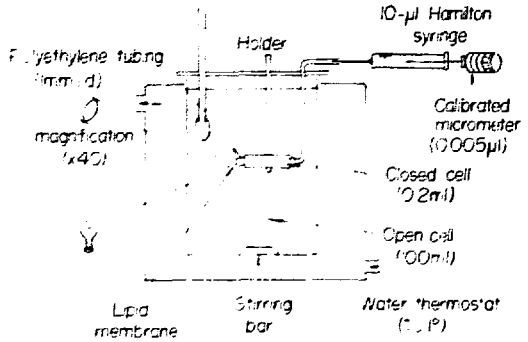


Fig. 1. Diagram of the apparatus for water permeability measurement.

and a closed 0.2 ml) compartment and the net volume flux produced by a NaCl gradient was measured. The membrane was generated from a solution of egg phosphatidylcholine (1%, w/v, in *n*-decane) in a medium of 0.3 molal NaCl pH 5.8 at 37 °C. At this temperature, the membrane usually turned black within less than 10 min, while longer periods were required at lower temperatures. The NaCl concentration of the open compartment was then raised to 0.6 molal, and flux measurements were conducted at 26 °C. A Hamilton syringe (No. 701) attached to the closed compartment and equipped with a calibrated micrometer allowed measurement of volume changes of 0.005 µl. The medium in the open compartment was continuously stirred with a magnetic stirrer. Vasopressin, Ca^{2+}, cyclic AMP or prostaglandin E_1 were added to this compartment following a permeability measurement of the untreated black membrane. The osmotic permeability coefficient, P_{os}, was calculated as described[15,16]. The permeability of the black membranes to ions was followed by measurements of the electrical resistance of the membrane as described[25], except that one of the compartments was constructed of a polyethylene tubing (2 mm internal diameter) through which an electrode was inserted.

Chemicals

Egg phosphatidylcholine was prepared essentially according to Pangborn[27]. It appeared as a single peak following thin-layer chromatography, using the solvents mixture of chloroform–methanol–water (65:25:4, v/v/v). The lipid was stored for not longer than 3 months, dissolved in chloroform, under Ar gas in sealed ampules at −18 °C.

Lysine-vasopressin (Lot 2309) was obtained from Sandoz, Basel. The concentration of the stock solution (about 1 mM) was determined spectrophotometrically[28]. Dilutions from the stock solution of the hormone were freshly made. Cyclic AMP was obtained from Sigma-Israel (Ramat Gan). Prostaglandin E_1 was obtained from the Upjohn Co., Kalamazoo, Mich.

RESULTS

Cyclic AMP and water permeability

In view of the stimulatory effect of vasopressin on water permeability of artificial lipid membranes[17,18], it was of interest to compare the potency of cyclic AMP and of vasopressin in this system. Table I shows that cyclic AMP over a wide concentration range had little or no effect on water permeability of the lipid membrane. In contrast, vasopressin at 10^{-10} M (5 μunits/ml) significantly increased the osmotic permeability coefficient, P_{os}, of the bilayer membrane[17]. The stimulatory effect of vasopressin was saturating at 10^{-9} M (50 μunits/ml).

Vasopressin activity affected by Ca^{2+}

Ca^{2+} was shown to inhibit the action of vasopressin on the water flow of the toad bladder[22,23]. The Ca^{2+} effect was completely reversed by increasing the vasopressin concentration[23].

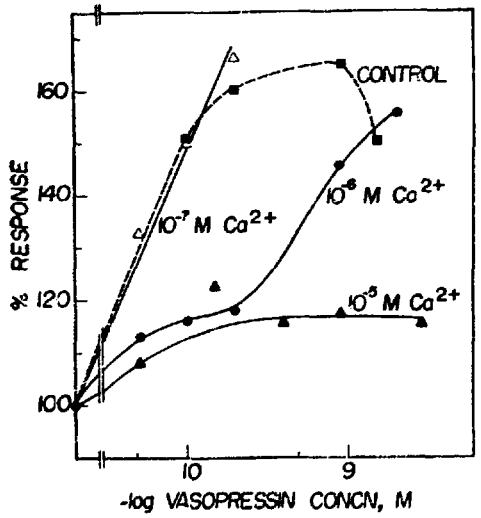
TABLE I

THE EFFECT OF CYCLIC AMP AND VASOPRESSIN ON WATER PERMEABILITY OF BILAYER LIPID MEMBRANES

Black membranes were generated at 37 °C, while volume flux as affected by cyclic AMP and vasopressin was measured[16,17] at 26 °C.

Addition		*P_{os}, mean ± S.E.** (μs^{-1})	*% of control*
None		25.8 (2)*	100
Cyclic AMP,	10^{-9} M	28.2 (2)	109
	10^{-8} M	27.6 (2)	108
	10^{-7} M	27.3 (2)	106
None		22.2 ± 0.9 (5)	100
Vasopressin,	10^{-10} M	30.6 ± 2.5 (5)	138
	$2 \cdot 10^{-10}$ M	37.4 ± 3.1 (5)	169
	10^{-9} M	34.2 ± 0.7 (5)	154

* Number of experiments in parentheses.



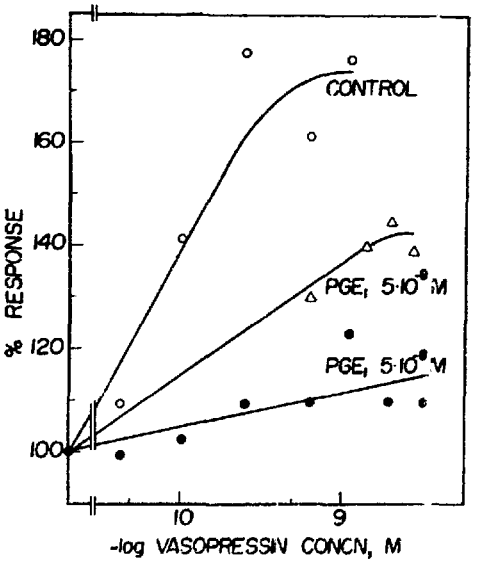


Fig. 2. Dose–response characteristics of action of vasopressin on osmotic water flow in bilayer lipid membranes as affected by Ca^{2+} concentration. Ca^{2+} was added prior to vasopressin.

Fig. 3. Dose–response characteristics of action of vasopressin on osmotic water flow in bilayer lipid membranes and inhibitory effect of prostaglandin E_1 (PGE_1). Prostaglandin was added prior to vasopressin.

Essentially the same observations can be reproduced in the model system (Fig. 2): Ca^{2+} at 10^{-6} M added to the medium prior to vasopressin inhibits the action of the antidiuretic hormone on water permeability. The effect of Ca^{2+} is apparently competitive, since it is overcome by increasing vasopressin concentration. Ca^{2+} at 10^{-7} M exerts no noticeable effect, while at 10^{-5} M the inhibitory effect is appreciable. Higher concentrations of vasopressin were not used, to avoid the concentration range in which the effect may be unspecific[17]. It is of interest that Ca^{2+} added at 10^{-7}–10^{-4} M subsequent to vasopressin showed no inhibitory effect.

Vasopressin activity affected by prostaglandin E_1

Prostaglandin E_1 was shown to inhibit the osmotic flow response of rabbit collecting tubules[3] and of toad bladder[20,21] to vasopressin. The inhibitory effect of prostaglandin E_1 could be overcome by high concentrations of vasopressin[21]. Using low concentrations of prostaglandin E_1 a similar phenomenon of apparent, competitive inhibition can be demonstrated in the model system (Fig. 3). Furthermore, as indicated for the effect of Ca^{2+}, this inhibitory effect of prostaglandin E_1 is only observed when added prior to vasopressin. Once the stimulatory effect of vasopressin on water permeability is apparent, subsequent addition of prostaglandin at the concentration range of 10^{-7}–10^{-4} M causes very little change.

Hormone-membrane complex formation

As the inhibitory effects of Ca^{2+} and prostaglandin E_1 in the model system are so critically dependent on the order of addition, it is possible that vasopressin forms a stable complex with the lipid membrane. When this complex is stabilized, Ca^{2+} and prostaglandin can no longer cause a measureable effect. If the hormone

were loosely bound to the membrane its effect on water permeability should decline as the medium to which vasopressin was added is diluted with the medium (0.6 molal NaCl). Fig. 4 illustrates an examination of the nature of the membrane–hormone complex: despite a 16-fold dilution of the medium, the original effect of the hormone is not diminished.

Vasopressin, cyclic AMP and ion permeability

It has been proposed[23] that vasopressin has two sites of action in epithelial membranes, one related to water transport, which is inhibited by Ca^{2+} and

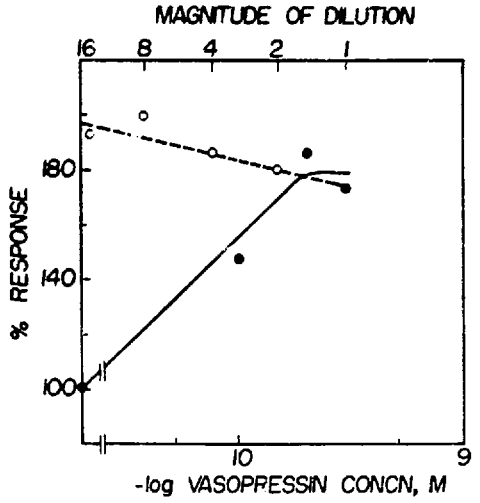


Fig. 4. Effect of vasopressin on water permeability of a bilayer lipid membrane (●—●) as affected by the magnitude of dilution of the medium in the open cell (○ – – – ○). The medium was diluted with 0.6 molal NaCl after the saturating effect of vasopressin was apparent.

TABLE II

ELECTRICAL RESISTANCES ACROSS BILAYER LIPID MEMBRANES AS AFFECTED BY VASOPRESSIN, NaCl GRADIENT AND VALINOMYCIN

Black membranes were generated in 0.3 molal NaCl at 37 °C, while the indicated additions of NaCl gradient and vasopressin, as well as the electrical measurements were performed at 26–27 °C. In Expt c the membrane was generated in 0.6 molal NaCl and 50 mM KCl.

Expt	*Addition*	*NaCl gradient (molal)*	*Resistance* $\Omega \cdot cm^2$
a	None	0	$1.3 \cdot 10^7$
	Vasopressin, $1 \cdot 10^{-9}$ M	0	$6.9 \cdot 10^6$
	$1.1 \cdot 10^{-6}$ M	0	$4.4 \cdot 10^6$
b	None	0	$1.1 \cdot 10^7$
	None	0.3	$7.9 \cdot 10^6$
	Vasopressin, $2.5 \cdot 10^{-9}$ M	0.3	$3.5 \cdot 10^6$
	$1.1 \cdot 10^{-6}$ M	0.3	$1.7 \cdot 10^6$
c	None	0	$6.9 \cdot 10^6$
	Vasopressin, 10^{-7} M	0	$5.3 \cdot 10^6$
	10^{-7} M + valinomycin 10^{-7} M	0	$2.2 \cdot 10^4$

prostaglandin E_1, and another side, related to Na^+ transport which is insensitive to Ca^{2+} and prostaglandin E_1. Two distinct adenylate cyclase enzymes were proposed[21], corresponding with the two hormone sites. In view of the direct effect of vasopressin on water permeability of the bilayer lipid membrane, a possible effect of vasopressin and of cyclic AMP on ion permeability of the lipid membrane was explored.

Table IIa shows that the permeability of the bilayer membrane to Na^+ or Cl^- was essentially unaffected by a wide range of vasopressin concentration. Four additional experiments supported this conclusion. The decrease in resistance by added vasopressin was within similar time courses. A marked drop in the electrical resistance of bilayer lipid membranes caused by vasopressin was recently reported[18], but the specificity of this effect is not clear, in view of the relatively high hormone concentration ($>10^{-5}$ M) used.

To simulate the conditions used for the measurements of water fluxes, a gradient of 0.3 molal NaCl was set between the two sides of the membrane, but again, the resistances recorded indicated no effect of vasopressin on ion permeability (Table IIb). The membrane, however, did respond to the ionophore valinomycin as expected[29,30]: in presence of K^+, valinomycin markedly reduced the resistance of the membrane (Table IIc). Table III shows that, similarly to vasopressin, cyclic AMP had no effect on the ion permeability of the bilayer. Valinomycin exerted the predictable effect on the electrical resistance in this experiment as well.

TABLE III

ELECTRICAL RESISTANCES ACROSS BILAYER LIPID MEMBRANES AS AFFECTED BY CYCLIC AMP AND VALINOMYCIN

The conditions were as described in Table IIc.

Addition	*Resistance* ($\Omega \cdot cm^2$)
None	$3.1 \cdot 10^7$
Cyclic AMP, 10^{-7} M	$2.2 \cdot 10^7$
10^{-6} M	$1.1 \cdot 10^7$
10^{-5} M	$2.2 \cdot 10^7$
10^{-5} M + valinomycin, 10^{-7} M	$3.8 \cdot 10^4$

DISCUSSION

The effect of vasopressin on water permeability of bilayer lipid membranes shows many similarities to the effect on biological membranes. (a) Sensitivity: relatively low quantities of the antidiuretic hormone are required to exert the effect[17]. The apparent high sensitivity of the lipid model system to vasopressin may be compared with the exquisite sensitivity of the isolated perfused collecting tubules of the rabbit[3], which responds to 0.25 μunit/ml. (b) Specificity: as shown earlier[17], oxytocin and a mixture of the amino acids comprising the vasopressin molecule could not substitute for vasopressin at comparable concentration. (c) Temperature dependence: the experimental activation energy of water transport was reduced in the presence of vasopressin

from 14 to 4 kcal·mole^{-1} (ref. 17), in agreement with the effect of the hormone on water permeability of toad bladder[31]. (d) Inhibition by Ca^{2+} of the vasopressin effect on water permeability in the model system (Fig. 2), and in biological systems[22,23]. (e) Prostaglandin E_1 inhibits at low concentration the water permeability of biological membranes[3,20,21] and of artificial lipid membranes (Fig. 3).

The competitive nature of the inhibitory effects by Ca^{2+} and prostaglandin E_1 (Figs 2 and 3), indicate the two types of interaction of the phospholipid membrane with vasopressin. One type is an electrostatic interaction between the lipid phosphoryl group with the positively charged group of the vasopressin molecule (lysine or arginine). Indeed, Ca^{2+} added after this interaction took place is not effective. Such interaction would also account for the much lower activity of oxytocin, comparing to vasopressin, in affecting water permeability of phospholipid membranes[17], or in releasing $^{45}Ca^{2+}$ from dicetyl phosphate monolayers[19]. The second interaction is probably hydrophobic, associated with the hydrocarbon phase of the bilayer structure. Prostaglandin E_1 (a fatty acid!) may interact with this "site" and thus inhibit the binding and/or the effect of vasopressin. It is also possible that prostaglandin E_1 interacts with vasopressin in solution, rendering it less effective. To differentiate between these possibilities we are currently studying the effect of prostaglandin E_1 on the binding of labeled vasopressin to liposomes.

The electrostatic and hydrophobic interactions between the vasopressin and the phospholipid molecules apparently lead to the firm binding of the hormone to the membrane as exhibited in Fig. 4. Utilizing another model system, the interaction of phospholipid dispersions (liposomes) with polypeptides, it has been concluded[32] that a phosphatidylserine–polylysine complex at neutral pH is stabilized by both electrostatic and hydrophobic interactions. The apparent formation of a vasopressin–phospholipid complex indicates that the quantity, rather than the concentration, of vasopressin is critical. The saturating quantity of vasopressin in our model system shows a stoichiometry of about 1 molecule vasopressin per 1.5 phospholipid molecules, provided that only the membrane lipid molecules are considered for rough estimation.

The increased water permeability of lipid membranes by vasopressin may be explained in terms of the permeation theory of Träuble[33], in which kinks are considered as carriers through lipid layers of the membrane. The kinks represent small mobile free volumes in the hydrocarbon phase of the membrane which could harbor water or larger molecules. In support of the theory it was reported that unsaturated compounds in bimolecular films of saturated long chain compounds lead to higher kink concentrations and favor a random distribution of the kinks[34]. Furthermore, the higher the degree of unsaturation of the hydrocarbon chains, the greater the water permeability of the lipid membranes[16,35]. We propose that the aromatic groups of phenylalanine and tyrosine, which are indespensable for vasopressin activity[36], protrude into the hydrophobic phase of the bilayer lipid membrane and increase the kink concentration and the water permeability. The electrostatic and hydrophobic interaction of the hormone with the membrane, discussed above, may be critical for the orientation of the hormone molecule in the membrane structure. The data support our conclusion[17] that vasopressin exerts a direct effect on the water permeability of the membrane, while the suggested mediator, cyclic AMP, is not active in the model system (Table I). Furthermore, vasopressin causes no significant change in the ion permeability of the lipid membrane (Table II). Thus, the bilayer lipid membranes apparently possess the

characteristics of the proposed site of action of vasopressin in biological membranes related to water transport[23], while the second site, related to a sodium transport, is lacking.

The effect of vasopressin on water permeability, but not on ion permeability, of the lipid membrane indicates distinct mechanisms for water and ion transport[23]. It may also reflect the disparity between the model and biological membranes, indicating the possible role of membrane proteins in controlling ion permeability. The conferral of ion permeability stimulation by vasopressin in a model system containing suitable proteins could be a promising tool in the reconstitution of biological-like membranes from individual components.

ACKNOWLEDGEMENTS

We wish to thank Dr F. de Körösy and Mrs M. Taboch for cooperation in the electrical measurements. The supply of prostaglandin E_1 by Dr J. E. Pike from the Upjohn Co., Kalamazoo, Mich., is appreciated. Valuable discussions with Dr Uriel Zor are acknowledged.

REFERENCES

1 Ussing, H. H. and Zerahn, K. (1951) *Acta Physiol. Scand.* 23, 110–127
2 Leaf, A., Anderson, J. and Page, L. (1958) *J. Gen. Physiol.* 41, 657–668
3 Grantham, J. J. and Orloff, J. (1968) *J. Clin. Invest.* 47, 1154–1161
4 Mazumo, F. and Edelman, I. S. (1971) *J. Clin. Invest.* 50, 1613–1620
5 Beck, N. P., Kaneko, T., Zor, U., Field, J. B. and Davis, B. B. (1971) *J. Clin. Invest.* 50, 2461–2465
6 Handler, J. S., Butcher, R. W., Sutherland, E. W. and Orloff, J. (1965) *J. Biol. Chem.* 240, 4524–4526
7 Chase, L. R. and Aurbach, G. D. (1968) *Science* 159, 545–547
8 Orloff, J. and Handler, J. S. (1964) *Am. J. Med* 36, 686–697
9 Robinson, G. A., Butcher, R. W. and Sutherland, E. W. (1971) *Cyclic AMP*, pp. 339–346, Academic Press, New York
10 Cuthbert, H. W. and Painter, E. (1968) *J. Physiol. London* 199, 593–612
11 Bangham, A. D. (1968) *Progr. Biophys. Mol. Biol.* 18, 29–95
12 Levine, Y. K. and Wilkins, M. H. F. (1971) *Nat. New Biol.* 230, 69–72
13 Hanai, T. and Haydon, D. A. (1966) *J. Theor. Biol.* 11, 370–382
14 Henn, F. A. and Thompson, T. E. (1969) *Annu. Rev. Biochem.* 38, 241–262
15 Price, H. D. and Thompson, T. E. (1969) *J. Mol. Biol.* 41, 443–457
16 Graziani, Y. and Livne, A. (1972) *J. Membrane Biol.* 7, 275–284
17 Graziani, Y. and Livne, A. (1971) *Biochem. Biophys. Res. Commun.* 45, 321–325
18 Fettiplace, R., Haydon, D. A. and Knowles, C. D. (1972) *J. Physiol. London* 221, 18P–20P
19 Kafka, M. S. and Pak, C. Y. C. (1969) *Biochim. Biophys. Acta* 193, 117–123
20 Orloff, J., Handler, J. S. and Bergstrom, S. (1965) *Nature* 205, 397–398
21 Lipson, L. C. and Sharp, G. W. G. (1971) *Am. J. Physiol.* 220, 1046–1052
22 Bentley, P. J. (1959) *J. Endocrinol.* 18, 327–333
23 Petersen, M. J. and Edelman, I. S. (1964) *J. Clin. Invest.* 43, 583–594
24 Graziani, Y. and Livne, A. (1972) *Israel J. Med. Sci.* 8, 1006
25 Cass, A. and Finkelstein, A. (1967) *J. Gen. Physiol.* 50, 1765–1784
26 Vreeman, H. J. (1966) *K. Ned. Akad. Wet. Proc. Ser. B* 69, 564–577
27 Pangborn, M. C. (1951) *J. Biol. Chem.* 188, 471–476
28 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* 193, 265–275
29 Mueller, P. and Rudin, D. O. (1967) *Biochem. Biophys. Res. Commun.* 26, 398–404

30 Andreoli, T. E., Tieffenberg, M. and Tosteson, D. C. (1967) *J. Gen. Physiol.* 50, 2527–2545
31 Hays, R. M. and Leaf, A. (1962) *J. Gen. Physiol.* 45, 933–948
32 Hammes, G. G. and Schullery, S. E. (1970) *Biochemistry* 9, 2555–2563
33 Träuble, H. (1970) *J. Membrane Biol.* 4, 193–208
34 Weiss, A., Spiess, J., Stuke, E. and Lagaly, G. (1972) *Z. Naturforsch.* 27b, 317–319
35 Finkelstein, A. and Cass, A. (1967) *Nature* 216, 717–718
36 Berde, B. and Boissonnas, R. A. (1968) in *Neurohypophysial Hormones and Similar Polypeptides* (Berde, B., ed.), pp. 802–870, Springer-Verlag, Berlin