VASOPRESSIN AND WATER PERMEABILITY OF ARTIFICIAL LIPID MEMBRANES

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SUMMARY

Vasopressin markedly stimulated the water permeability of bilayer lipid membranes: a two-fold increase was measured at 25° in presence of $1.7 \cdot 10^{-9}$ M (50 μunits/ml) vasopressin. Oxytocin and a mixture of the amino acids comprising the vasopressin molecule could not substitute for vasopressin at comparable concentration. The experimental activation energy of water transport was reduced in the presence of vasopressin from 14 to 4 kcal/mole, in agreement with the effect of the hormone on water permeability of toad bladder.

Vasopressin, the antidiuretic hormone, increases water flow and sodium transport along osmotic gradients. Frog skin and toad bladder were commonly used for studying the biological effects of vasopressin, and several proposals for the mode of action of the hormone were advanced (1,2,3,4). Owing to the complexity of the biological membrane, it is not surprising that the mechanism of the vasopressin action is not clear. The availability of an experimental system for studying bilayer lipid membranes (5,6) and the similarity of such model membranes to biological membranes (7,8) focused our attention on the water permeability of the lipid membranes as affected by vasopressin. This communication describes a stimulatory effect of the hormone on osmotic water flux across artificial lipid membranes. The stimulation bears similarities to the biological effect of vasopressin. An abstract of part of this report has appeared (9).

MATERIALS AND METHODS

The osmotic water flux across thin lecithin membranes was measured accor-

ding to described procedures (10,11). In principle, a thin lipid membrane was formed between an open (100 ml) and a closed (0.2 ml) compartment and the net volume flux produced by a NaCl gradient was measured. The thin membrane was generated from a solution of egg phosphatidyl choline (1%, w/v in n-decane) in a medium of 0.3 molal NaCl at 37°. At this temperature, the membrane usually turned black within less than 10 minutes, while longer periods were required at lower temperatures. The NaCl concentration of the open compartment was then raised to 0.6 molal. A Hamilton syringe (#701) attached to the closed compartment and equipped with a calibrated micrometer allowed measurement of volume changes of 0.005 µl. The sequence of temperature change(s) and the addition of vasopressin to the open compartment was as indicated in Fig. 1. P_{os}, the osmotic permeability coefficient, was computed from the linear plots of volume changes according to Price and Thompson (11).

Egg phosphatidyl choline was prepared as described (12). The following vasopressin preparations were used: a) L-8, lot 67018 from Sandoz, Basel; b) L-8, lot 60C-3410 from Sigma-Israel, Ramat Gan; c) Pitressin, lot 344551 from Parke, Davis & Co., Detroit, Mich. Oxytocin ("Synthocinon"), lot 67091 was obtained from Sandoz, Basel. The peptide concentration was determined according to Lowry et al (13), using tyrosine as standard.

RESULTS AND DISCUSSION

The water permeability of lecithin bilayer membrane is temperature dependent (11,14). Fig. 1 substantiates this observation and shows that vasopressin added at $1.7 \cdot 10^{-9}$ M (50 µunits/ml) increases the water permeability of the lipid membrane. The increased rate of water movement could be discerned within 2 minutes following the addition of vasopressin. The membranes were commonly stable for over 2 hours, facilitating measurement of the effect of several vasopressin concentrations on the same membrane. Fig. 2 shows that the hormone is effective in promoting water permeation of lipid membranes at concentrations close to the physiological range (4).

Stock solutions of vasopressin diluted in distilled water and kept at 4°

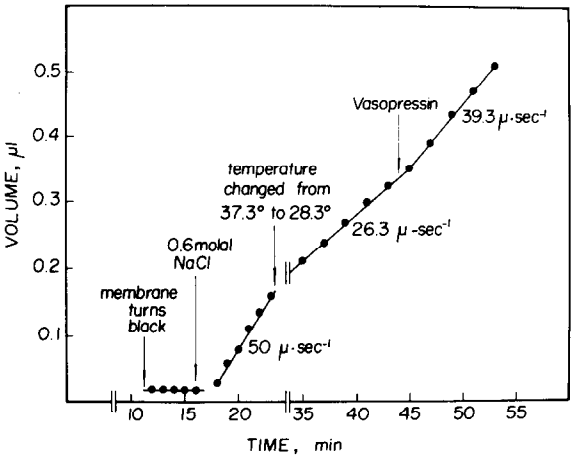

Fig. 1. Water permeability of a thin phosphatidyl choline membrane as affected by temperature and vasopressin ($1.7 \cdot 10^{-9}$ M, 50 µU/ml). Membrane formation was taken as zero time. Values of the osmotic permeability coefficient, expressed in units of $\mu \cdot sec^{-1}$, are included.

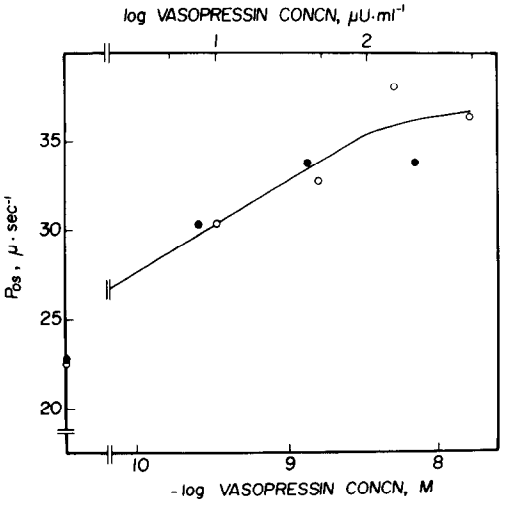

Fig. 2. Effect of vasopressin concentration on P_{os}, the osmotic permeability coefficient, of thin phosphatidyl choline membranes. The results of two experiments are included. Temperature: 31.7°.

lost gradually their activity of promoting membrane permeability, activity declining by about 50% every 36 hours. The use of freshly diluted preparations taken from sealed ampules assured the reproducibility of vasopressin activity in the model system.

A vasopressin preparation showing effective antidiuretic activity on human patients with diabetes insipidus (Prof. G.M. Berlyne, The Central Negev Hospital, Beersheva, personal communication), was simultaneously analyzed in our system and found effective in elevating the water permeability of lecithin membranes. An older preparation (origin unknown), which had a questionable antidiuretic activity, was also found ineffective with respect to water movement in the lipid system.

An equimolar mixture of the 8 amino acids comprising the vasopressin molecule (cystine, tyrosine, phenylalanine, glutamine, asparagine, proline, lysine and glycine) added at 10^{-9} to 10^{-8} M exerted no effect on the water permeability of the artificial membranes. A 13% increase of water movement was measured following the addition, at 10^{-7} M, of the mixture of the 8 amino acids or of bovine serum albumin. In contrast, vasopressin added at $1.7 \cdot 10^{-9}$ M increased the water permeability of the lipid membranes by up to 250%, depending on the temperature. An oxytocin preparation, which was biologically active in promoting milk ejection in rats, did not stimulate water transport in the model system when added at 10^{-9} M (100 μinternational units/ml). A stimulation of 10% and 20% of water transport were apparent in presence of $5 \cdot 10^{-9}$ M (500 μIU/ml) and $5 \cdot 10^{-8}$ M (5000 μIU/ml) oxytocin, respectively. This indicates a striking specificity in the effect of the vasopressin on water permeability of bilayer lipid membranes. The availability of a great number of synthetic analogues and homologues of the neurohypophysial hormones (15), allows an extensive study of structure-function relationship with respect to the water permeability-promoting activity in the model system.

Fig. 3 shows that vasopressin changes significantly the temperature dependence of P_{os}. The experimental energy of activation (E_a) of the osmotic permeability coefficient computed from Fig. 3 is decreased from 14.3 kcal/mole to a value of 4.0 kcal/mole following the addition of 50 μU/ml of vasopressin. A decrease in E_a of water permeability from 9.8 to 4.1 kcal/mole due to vasopressin was already reported for toad bladder (1). Furthermore, in both the bio-

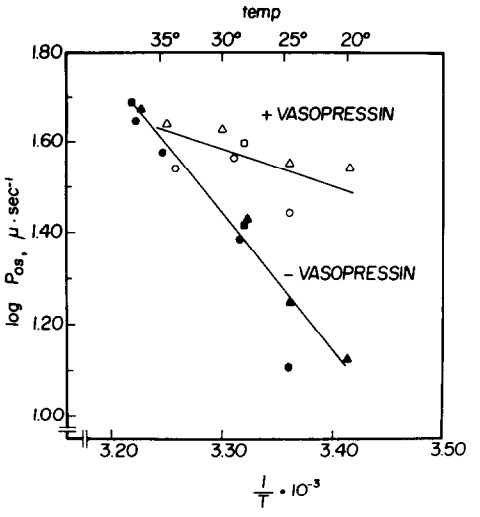

Fig. 3. Temperature dependence of water permeability of thin phosphatidyl choline membranes as affected by vasopressin ($1.7 \cdot 10^{-7}$ M, 50 μU/ml). The logarithm of P_{os} is plotted versus the reciprocal of absolute temperature.

logical system (1) and in the model system, the effect of vasopressin on water permeability was hardly noticeable at temperatures close to 37°.

It has already been shown that lipid bilayer membranes are similar to cellular membranes with respect to thickness, electrical capacitance, interfacial tension (16), permeability to urea, glycerol and mannitol (17), as well as water permeability and activation energy of water permeation (6,14). The present data further emphasize the similarity of the bilayer and the biological membranes, and point to some interesting implications. For example, cyclic 3',5'-AMP (cAMP) has been suggested to function as an intracellular mediator of the effect of vasopressin (18,19). Conversely, it has been claimed that the effect of vasopressin on cell membrane permeability is independent of cAMP (20). The direct action of vasopressin in the bilayer lipid membranes supports the latter claim. The model system employing artificial lipid membranes could further assist in elucidating the mode of action of vasopressin on molecular level.

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