SURFACE ACTIVITY OF VASOPRESSIN AND ITS INTERACTION WITH ARTIFICIAL AND BIOLOGICAL MEMBRANES

G. V. Ostrovskaya, S. I. Shevchenko, B. R. Mogilevich, R. V. Rybal'chenko, and V. K. Rybal'chenko

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The physiological action of the neurohypophyseal hormone vasopressin on the cell is mediated through the plasma membrane. It is suggested that its specific receptors (of the V_1 or V_2 type), located in the membrane, transmit the hormonal signal to intracellular structures [10, 13]. Intracellular processes taking place after activation of vasopressin receptors have been studied in sufficient detail [9]. However, according to our data [6, 8] and our previous hypothesis [7], regulatory peptides can interact with the lipid matrix of the membrane, leading either to further binding with the receptor or to realization of other effects (an increase in ionic permeability of membranes, direct activation of G-proteins, etc.).

In this investigation the surface activity of vasopressin (VP), and its action on liposomes, monolayers formed from the plasma membrane (PM) fraction of small intestinal myocytes, and vesicles of the sarcoplasmic reticulum (SR) of skeletal muscles, on which no specific vasopressin receptors have been found, were analyzed.

EXPERIMENTAL METHOD

Surface activity of the hormone and its interaction with monolayers of plasma membranes were estimated by measuring the change in two-dimensional pressure and the boundary potential step [12, 14]. Membranes of SR and plasma membranes of myocytes were isolated as described in [4, 5]. Liposomes were obtained by the method in [15]. Ca^{2+} transport was determined as fluorescence of chlortetracycline [11]. The action of VP was assessed in relation to permeability of closed membrane vesicles for Ca^{2+}. For this purpose the efficiency of calcium transport, ATPase activity (vesicles of SR), and the Ca^{2+} concentration in the internal space of the liposomes were investigated. Efficiency of calcium transport (the ratio Ca^{2+}/ATP) is the quantity of Ca^{2+} transported by one Ca-ATPase molecule through the membrane as a result of hydrolyis of one ATP molecule [1, 3].

EXPERIMENTAL RESULTS

Since cell membranes are interphase boundaries, interaction of biologically active compounds with them is largely determined by the surface-active properties of these substances. In this connection, we considered it necessary to study surface activity of VP and its ability to interact with monolayers formed from the plasma membrane fraction of small intestinal myocytes, for which the problem of the existence of specific receptors for this peptide has not been finally settled.

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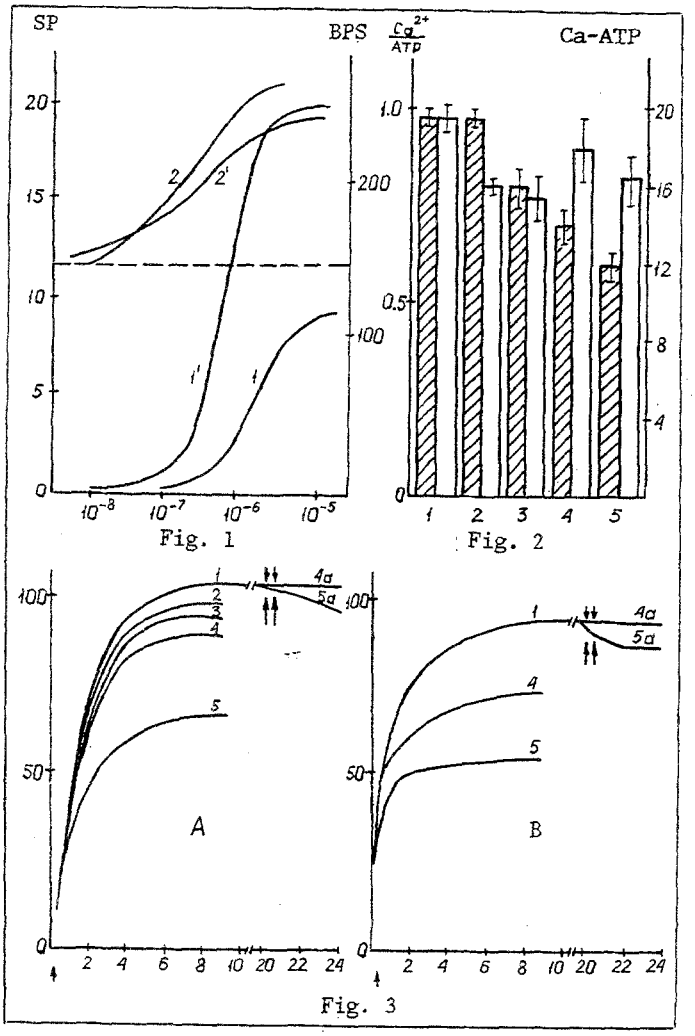

Fig. 1. Changes in surface pressure and boundary potential step during adsorption of VP on free surface of 0.01 M KCl (1 and 1′ respectively) and in presence of a monolayer of myocyte PM on the phase boundary (2 and 2′ respectively).

Fig. 2. Efficiency of Ca^{2+} transport (shaded columns) and Ca-ATPase activity (unshaded columns) of preparations of sarcoplasmic reticulum vesicles in presence of vasopressin: 1) 0 M; 2) 0.1 mN, 3) 10 nM, 4) 1 μM, 5) 0.1 μM.

Fig. 3. Change in intensity of fluorescence of CTC in vesicles of light fraction of sarcoplasmic reticulum (A) and in liposomes (B) in presence of vasopressin. Arrow indicates addition of suspension of vesicles (150 μg protein) A, of suspension of liposomes (50 μg) – B. 1) 0 M, 2) 10 nM, 3) 1 μM, 4) 0.1 mM, 5) 1 mM (a – peptide added at point indicated by two arrows). Abscissa, time (in min); ordinate, change in intensity of fluorescence (in %).

Graphs of dependence of surface pressure (SP) and boundary potential step (BSP) during adsorption of VP from the subphase on the clean electrolyte surface (0.01 M KCl) and on the surface filled with plasma membrane monolayer, are shown in Fig. 1. Initial changes in the values of SP and BPS were observed when the subphase contained 0.1 μM of the peptide, but when the VP concentration reached 1.0 μM a spread out monolayer was formed on the electrolyte–air partition boundary. Maximal values of SP (9.8-10.0 mN/m) and BPS (237-240 mV), characterizing a sufficiently dense absorption layer of VP, were reached with the peptide in concentrations above 10 μM. The steady state was established in the system in the course of 30-40 min.

The primary changes in parameters of the monolayer formed from the PM fraction of smooth muscle cells of the small intestine (initial parameters of the monolayer – SP = 12.0 mN/m and BPS = 145-150 mV) were produced by VP in concentrations of the order of 10 nM (Fig. 1). If the concentration of the peptide in the subphase was 5 μM, however, SP of the monolayer was almost doubled. The time taken to establish a steady state in the system in this case was 10-15 min.

The results are evidence that, due to its surface-active properties, VP is adsorbed, and later inserted into the monolayer made from the PM fraction. Under these circumstances the area occupied by a molecule of the peptide in the monolayer at maximal adsorption is 1.0-2.5 nm^2, depending on the original density of the PM monolayer.

Processes taking place following modification of the proteolipid monolayers by the peptide may also take place during the action of VP on biological and artificial bilayer membrane structures. For instance, the efficiency of Ca^{2+} transport by SR vesicles falls under the influence of VP (Fig. 2). In this case the concentration dependence is exponential in character within the concentration range studied. The decrease in Ca^{2+}/ATP indicates either partial inactivation of Ca-ATPase or Ca^{2+} antiport from the SR vesicles as a result of increased membrane permeability for them. In order to discover the causes of this effect we analyzed the effect of VP on hydrolytic activity of Ca-ATPase relative to ATP. The data in Fig. 2 are evidence that VP, within the concentration range tested, has virtually no effect on Ca-ATPase activity. Consequently, the decrease in efficiency of Ca^{2+} transport in this experiment was not dependent on Ca-ATPase activity, and a fall in Ca^{2+}/ATP was due to an increase in permeability of the SR membranes under the influence of VP.

To determine the location of the site of Ca^{2+} leakage through the membrane, we used chlortetracycline (CTC) to study the Ca^{2+} concentration in calcium-loaded phosphatidylcholine liposomes and SR vesicles in the presence of VP. CTC was used because the intensity of its fluorescence depends on the Ca^{2+} concentration in the immediate vicinity of the hydrophobic region of the membranes [2]. As will be clear from Fig. 3, an increase in the VP concentration causes a decrease in fluorescence of CTC both in SR vesicles and in liposomes, evidence of a fall in the Ca^{2+} concentration in these structures. Comparison of the curves in Fig. 3a and b, shows that VP equally increases permeability both of liposomal membranes and of SR membranes. Thus under the experimental conditions used VP causes an equivalent increase in permeability of the lipid bilayer of the membranes for calcium ions.

Incidentally, an essential factor in these experiments is the distribution of VP relative to the membrane. In all the experiments whose results were given above the membranes were introduced into a medium which already contained VP. This method does not rule out transmembrane transport of VP under the influence of osmotic shock (as a result of differences in the ionic composition of the medium used to store the membrane vesicles and the medium used to record Ca^{2+} transport), with subsequent localization of the peptide in the internal space of the closed membrane structures or in the hydrophobic lipid matrix. If VP was introduced into a medium already containing liposomes or SR vesicles, the change in Ca^{2+} concentration inside the membrane vesicles was very small. With this method of introduction the peptide did not undergo transmembrane transport, for at that moment the composition of the outer and inner median of the membrane vesicles was balanced, and the peptide was located mainly on the outer side of the membrane.

The action of VP under these conditions evidently leads to very slight changes in the structural organization of the lipid bilayer. It may perhaps be limited to the outer monolayer. Local changes of this kind cannot lead to any substantial increase in membrane permeability If VP was localized on both sides of the membrane, the structure of both outer and inner monolayers was altered, leading ultimately to release of Ca^{2+} from the closed membrane vesicles.

Thus VP causes an increase in permeability of the phospholipid bilayer of the membranes for Ca^{2+}, which is evidently determined by the distribution of the peptide in both inner and outer compartments of the membrane vesicles.

It can be concluded from these experiments that VP can interact with the lipid matrix of biological membranes. This interaction is determined by the surface-active properties of the peptide As a result the hormone is inserted into the lipid bilayer (irrespective of whether a specific receptor is present or not) and influences its physicochemical properties, which may be manifested as an increase in permeability of the lipid-bilayer of the membrane for Ca^{2+}.

REFERENCES

1. A. I. Boldyrev and V. I. Mel'gunov, Transport ATPases, Progress in Science and Technology. Series: Biophysics, Vol. 17 [in Russian], Moscow (1985).
2. G. E. Dobretsov, Fluorescent Probes in the Study of Cells, Membranes, and Lipoproteins [in Russian], Moscow (1989).
3. V. B. Ritov, Biokhimiya, **36**, No. 3, 393 (1971).
4. V. B. Ritov, V. I. Mel'gunov, P. G. Komarov, et al., Dokl. Akad. Nauk SSSR, **233**, No. 3, 730 (1977).
5. V. K. Rybal'chenko, P. V. Pogrebnoi, T. G. Gruzina, et al., Byull. Éksp. Biol. Med., **97**, No. 1, 106 (1984).
6. V. K. Rybal'chenko, G. V. Ostrovskaya, and N. E. Kucherenko, Byull. Éksp. Biol. Med., **102**, No. 12, 681 (1989).
7. V. K. Rybal'chenko, Dokl. Akad Nauk SSSR, **314**, No 4, 984 (1990).
8. V. K. Rybal'chenko and B. R. Mogilevich, Dokl. Akad Nauk Ukr. SSR, Ser B, No. 10, 66 (1990).
9. J. Tepperman and H. Tepperman, Physiology of Metabolism and of the Endocrine System [in Russian], Moscow (1989).
10. V. G. Shalyapina (ed.), Physiology of Hormonal Reception [in Russian], Leningrad (1986).
11. S. I. Shevchenko, T. L. Chernova, E. V. Men'shikova, et al., Biokhimiya, **54**, No. 8, 1526 (1989).
12. J. J. Bickerman, Surface Chemistry for Industrial Research, New York (1948).
13. P. Crause, R. Boer, and F. Fahrenholz, FEBS Lett., **175**, No. 2, 383 (1984).
14. G. Gabella, Experientia, **28**, No. 2, 948 (1972).
15. G. Meissner and S. Fleischer, J. Biol. Chem., **249**, No. 1, 302 (1974).