INTERACTION OF OXYTOCIN WITH LIPIDS OF ARTIFICIAL AND BIOLOGICAL MEMBRANES

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As was shown previously [3], oxytocin is a specific blocker of transport Mg,Ca-ATPase and of ATP-dependent Ca^{++} transport in the vesicular fraction of the plasma membranes (PM) of smooth-muscle cells of the rabbit small intestine. The effect of oxytocin is independent of whether it is added to the tissue homogenization medium or to the incubation medium of the vesicular fraction of PM. Since we measured both ATPase activity and ATP-dependent Ca^{++} transport on membrane vesicles oriented with the cytoplasmic side toward the incubation medium, the blocking effect of oxytocin on these two processes cannot be explained from the standpoint of the recently asserted view [7] that the hormone molecule interacts directly with a membrane receptor on the outer surface of PM.

We accordingly postulated that the primary process of oxytocin binding with PM of smooth-muscle cells is interaction of the hormone molecule with lipid molecules of the membrane matrix. Experimental data confirming this hypothesis are given in this paper.

EXPERIMENTAL METHOD

The PM fraction was isolated from smooth-muscle tissue of the rabbit small intestine by differential centrifugation in a sucrose density gradient, it was characterized with respect to marker enzymes and by electron microscopy, and interaction between oxytocin and lipids was recorded by the monolayers method [1, 4].

EXPERIMENTAL RESULTS

As will be clear from Fig. 1, addition of oxytocin to the electrolyte in a concentration of 1.33×10^{-6} M did not change the surface pressure, but it was accompanied by a sharp increase in the boundary voltage step (BVS) which, in certain cases, was observed with the hormone in a concentration of 10^{-8} M. A further increase in oxytocin concentration led to a rise of surface pressure, and at 5×10^{-6} M the system reached its steady-state value after 35-40 min, with the formation of a monolayer with surface pressure of about 10 mN/m and BVS of +155 mV. The value of adsorption (G) for the oxytocin monolayer, namely 3.1×10^{-6} M/m^2, can be obtained by Gibbs' equation: $G = 1/RT \cdot d\pi/d \ln C$ (Fig. 1a).

Compression-expansion isotherms of the oxytocin monolayers indicate that the hormone molecules are actively concentrated on the phase boundary between electrolyte solution and air, but do not form monolayers as elastic as, for example, phospholipids [1]. If the latter are present on the surface of the electrolyte solution, hormone molecules interact with molecules of the lipid monolayer even in concentrations so low that no oxytocin monolayer is formed. Under these circumstances the constant surface pressure was reached in the course of 20-30 min and the level of oxytocin adsorption was 5.13×10^{-7} M/m^2 during interaction with "loose" and 2.88×10^{-7} M/m^2 during interaction with "dense" azolectin monolayers. The area of the monolayer available for the inserted hormone molecule was 3.24 and 5.76 nm^2. BVS for interaction of oxytocin with azolectin monolayers also was increased (Fig. 1b) up to

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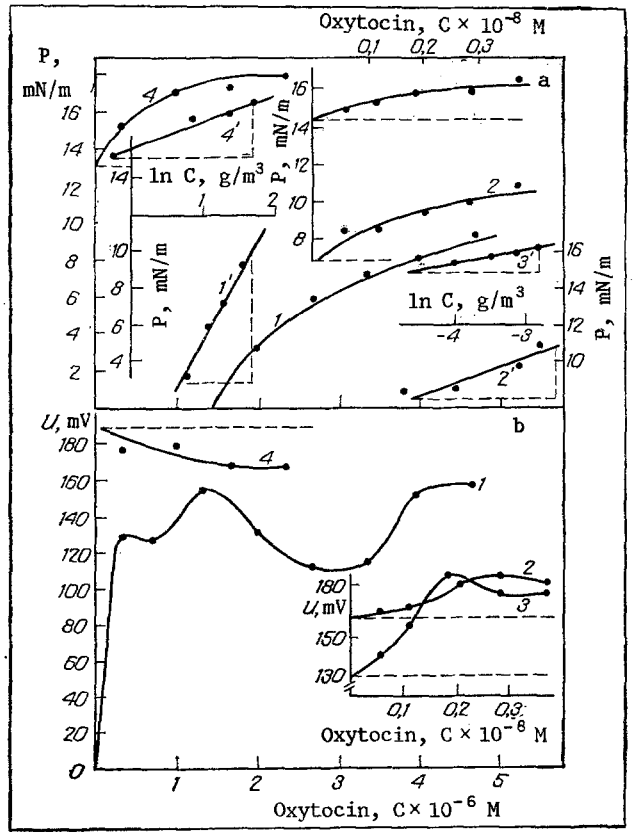

Fig. 1. Changes in surface pressure (a) and BVS (b) on addition of oxytocin to substrate phase. 1) Unoccupied; 2, 3) electrolyte surface occupied by azolectin monolayers (0.01 M KCl, pH 7.2, 22°C); 4) monolayer of PM fraction. 1'-4') Calculated values of hormone adsorption on phase boundary.

180 mV, despite differences in its initial values. It follows from these data that the lipid monolayer acts as "primer" for oxytocin, enabling the insertion of hormone molecules into the monolayer even if present in low (10^{-9} M) concentrations in the solution. Under these circumstances oxytocin is inserted into a "loose" monolayer more actively than into a "dense" monolayer.

On interaction of oxytocin with monolayers formed from the PM fraction, an increase of surface pressure and decrease on BVS was observed when the hormone concentration was as low as (5-8) × 10^{-9} M. With an increase in concentration of the hormone in the substrate phase (10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6} M) these characteristics reached constant values after successively shorter time intervals (40, 30, 10, and 5 min). The level of oxytocin adsorption was constant at 5.56 × 10^{-7} M/m² (close to values of adsorption of the hormone during interaction with azolect n monolayers), and the area per peptide molecule was about 3 nm².

These results are evidence that oxytocin can insert itself into the lipid matrix of PM on account of its characteristic surface activity. The very small increase in adsorption of the hormone on monolayers formed from the PM fraction (compared with azolectin monolayers), like the decrease in area per peptide molecule, can be taken as evidence that a certain fraction of oxytocin interacts with the membrane through its protein components. Through interaction with membrane lipids, the oxytocin molecule evidently acquires a conformation in which it is capable of insertion into the lipid matrix, and subsequent binding with a receptor (or ATPase molecule?), as a result of which the hormone inhibits ATP-dependent Ca^{++} transport by the PM fraction [5], whatever the orientation of the membrane vesicles. This conformation of the oxytocin molecule can perhaps be acquired spontaneously also in an aqueous medium. However, the probability of such a transition is very low, as is shown by the very small increase in adsorption of the hormone on monolayers composed of the PM fraction compared with azolectin monolayers.

TABLE 1. Effect of Liposomes Preincubated with Oxytocin on Mg,Ca-ATPase Activity of Plasma Membrane Fraction

Modifiers: lipids 300 μg/ml; oxytocin 10^{-6} M	ATPase activity (mean values of 6-8 measurements, per cent)	
	medium without calmodulin	medium with calmodulin, 25 μg/ml
Without modifiers	100	210
Azolectin	160	250
Azolectin + oxytocin	0,0	0,0
Phosphatidylcholine	145	250
Phosphatidylcholine + oxytocin	0,0	0,0
Azolectin + cholesterol: 150/150 μg in 1 ml	120	190
Azolectin + cholesterol + oxytocin	0,0	0,0
Total bovine brain lipids	60	160
Total lipids + oxytocin	0,0	0,0
Supernatant after sedimentation of liposomes	90	205

Legend. Mg,Ca-ATPase determined as difference between total activity in medium containing 100, 20, and 5 mM sodium, potassium, and magnesium chlorides, 3 mM ATP, 5 mM imidazole-HCl, and 100 μM $CaCl_2$ (pH 7.2; 37°C) and activity in medium of the same composition, but containing 100 μM oxytocin. Liposomes were incubated for 30 min in medium for determination of total ATPase activity, but without ATP.

To test this hypothesis, experiments were carried out to study the effect of azolectin liposomes, preincubated with oxytocin, on the transport Mg,Ca-ATPase of the PM fraction of smooth muscle cells. The initial assumption was that if oxytocin binds with lipids of liposomes, but if vesicles of PM and liposomes are incubated they interact and fuse with one another [4], activity of the enzyme must decline. As Table 1 shows, liposomes of different composition preincubated with 10^{-6} M oxytocin for 30 min, block Mg,Ca-ATPase irrespective of the presence or absence of calmodulin, an activator of this particular activity. It follows from these results that blockage of ATPase is the result of the action of liposome-bound oxytocin, for the supernatant after sedimentation of the liposomes (Table 1) did not change the enzyme activity. Oxytocin, at least in a concentration of 10^{-6} M, likewise did not exhibit specificity toward lipids. On interacting with lipids of liposomes, the oxytocin molecule evidently acquires a certain conformation (as has been shown, for example, for ACTH [6]), which is able to exhibit the inhibitory effect of the hormone. Support for this view is given by the results of investigations into the formation of ionic channels in phosphatidylserine bilayers in the presence of oxytocin [2].

The primary process of binding of oxytocin with the membrane and manifestation of its inhibitory effect on the plasma membrane Mg,Ca-ATPase of smooth mucle cells is therefore interaction of the hormone molecule with molecules of membrane lipids through the characteristic surface activity of the peptide.

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