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ROLE OF HYDROPHOBIC COMPONENTS OF THE PLASMA MEMBRANE IN THE RESPONSE OF FROG URINARY BLADDER CELLS TO ANTIDIURETIC HORMONE

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Interaction between antidiuretic hormone (ADH) and its receptor located in the basal plasma membrane of the cell is accompanied by cyclic AMP (cAMP) formation and culminates in molecular restructuring of the apical plasma membrane and increased permeability for water [8]. The role of hydrophobic membrane components in realization of the effect of ADH has not hitherto been studied, although the state of the hormone receptor, of adenylate cyclase, and also reorganization of the water channels and the formation of aggregates of particles in the apical membrane could be dependent on lipids. It has recently been shown that exogenous lipids can potentiate the action of ADH [5].

The aim of this investigation was to study the functional role of hydrophobic interaction in the plasma membranes of epithelial cells in response to ADH and to an increase in the flow of water along the osmotic gradient. For this purpose, the detergent Triton X-100 was applied to the isolated frog urinary bladder and changes in water transport in response to ADH were recorded.

EXPERIMENTAL METHOD

The investigation was conducted on spring frogs (*Rana temporaria* L.). Water transport was studied in the isolated urinary bladder by a gravimetric method [3]. Ringer's solution diluted 1:10 with water was introduced into the bladder from the side of the mucous membrane, and undiluted Ringer's solution was applied from the side of the serous membrane. The following reagents were used: Triton X-100 (Ferak, West Berlin), theophylline (Richter, Hungary), forskolin (Calbiochem, USA), and cAMP (Reanal, Hungary); the source of the ADH was pituitrin P (Kaunas Endocrine Preparations Factory). The sodium and potassium concentrations were measured on a Zeiss III frame photometer in an air-propane flame.

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TABLE 1. Effect of Triton X-100 on Permeability for Water and Ions Under the Influence of 2 IU/ml of ADH (M ± m)

Experimental conditions	Site and period of action of detergent	Absorption of water, μl /cm²/min			Concentration of electrolytes, mM	
		I	II	III	Na	K
ADH (7)	—	0,089±0,015	1,15±0,18	0,85±0,13	19,5±1,9	1,47±0,34
Forskolin 5×10⁻⁶ M (8)	—	0,070±0,015	2,84±0,34	1,62±0,25	—	—
Triton X-100	Serous membrane					
10 mg/ml (9)	II, III	0,073±0,022	0,59±0,072	0,43±0,029	—	—
0.1 mg/ml (9)	II, III	0,047±0,012	0,052±0,008	0,042±0,007	—	—
The same + ADH (9)	II, III	0,042±0,007	0,048±0,007	0,084±0,019	20,7±2,8	0,88±0,1
Triton X-100	Mucous membrane					
0.1 mg/ml (9)	I, II, III	0,051±0,008	0,11±0,03	0,094±0,026	—	—
The same + ADH (9)	I, II, III	0,094±0,026	0,11±0,02	0,13±0,02	21,1±2,1	0,61±0,07
The same + forskolin (7)	I, II, III	0,150±0,05	0,36±0,09	0,35±0,07	—	—

Legend: Concentration in Ringer's solution (in mM): Na 108 mM, K 2.9 mM; in hypotonic solution 11 and 0.3 mM, respectively. I, II, III) Consecutive 30-min periods of investigation of permeability of frog urinary bladder wall; ADH and forskolin applied from side to serous membrane during periods II and III. Number of experiments given in parentheses.

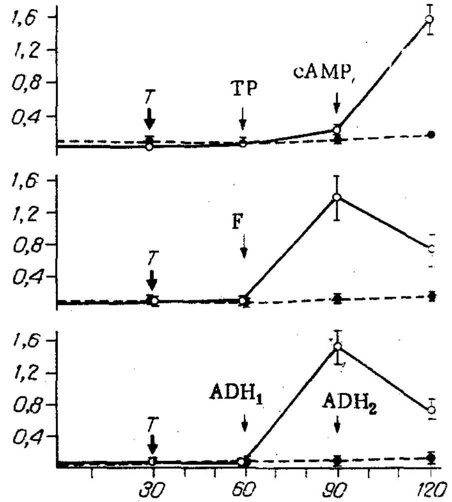

Fig. 1. Effect of Triton X-100 (T) on reaction of frog urinary bladder cells to theophylline (TP) with cAMP, ADH, and forskolin (F). Broken line — experiments in which the following substances were added to the solution on the serous membrane side: Triton X-100 (0.1 mg/ml), ADH_1 (2 IU/ml), ADH_2 (10 IU/ml; only to the solution in which Triton X-100 was dissolved). Continuous line — addition of ADH, TP (1 mM), cAMP (2 mM) or F (5 μM) in experiments on control bladders. Here and in Figs. 2 and 3: Abscissa, time (in min); ordinate, flow of water along osmotic gradient (1 μl/cm²/min).

EXPERIMENTAL RESULTS

Triton X-100 applied from the side of the serous membrane in a concentration of 10 mg/ml, used for solubilization of membranes [2, 7], increased the permeability of the bladder wall for water and ions, probably because of a marked change in the physicochemical properties of the epithelium (Table 1). Experiments were carried out with the addition of lower concentrations on the side of the serous and mucous membranes. When the Triton X-100 concentration was 100 times lower than that mentioned above, permeability for water and sodium was not increased (Table 1). It can be postulated that in the presence of such a low concentration of Triton X-100 changes in hydrophobic interaction in the membrane would be detectable only after restructuring the membranes under the influence of ADH. In fact, Triton X-100 in the solution applied to the serous membrane prevented the ADH-induced increase of permeability for water, and increasing the ADH concentration was ineffective (Fig. 1). Triton X-100 prevented the action of ADH also after the hormone had achieved its maximal effect (Fig. 2). A change in the character of hydrophobic interaction in the basolateral plasma membranes thus abolishes the response of the cell to ADH. This could depend not only on a change in interaction between hormone and receptor, but also on disturbance of signal transmission to adenylate cyclase and to an influence on the functional state of this enzyme.

The substance forskolin is known to induce maximal activation of adenylate cyclase, bypassing the ADH receptor [6]. In the present experiments, forskolin was added to one fraction of the frog urinary bladder, and Triton X-100 initially, followed by forskolin, was added to another fraction of the bladder from the same frog. The action of forskolin was depressed compared with that in experiments with intact urinary bladders, to which only forskolin was added without Triton X-100 (Fig. 1). It may thus be tentatively suggested that the

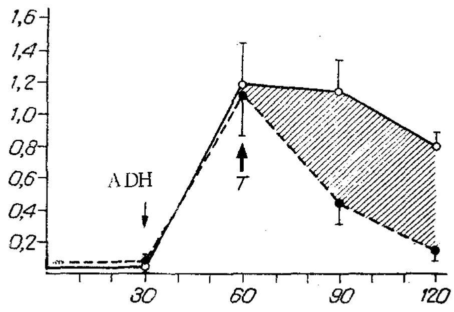

Fig. 2

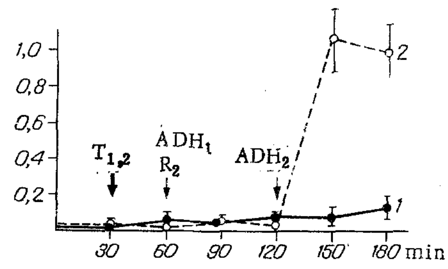

Fig. 3

Fig. 2. Depression of response to ADH on addition of Triton X-100 at peak of ADH effect. Continuous line — action of 2 IU/ml ADH, broken line — Triton X-100 (T) added in a dose of 0.1 mg/ml at peak of action of hormone.

Fig. 3. Reversibility of action of Triton X-100. Addition of 0.1 mg/ml of Triton X-100 (T) to solution in contact with serous membrane of urinary bladder. Continuous line — addition of 2 IU/ml ADH (ADH_1) in the presence of Triton X-100 (1), broken line — washing out Triton X-100 with Ringer's solution (R_2) and addition of 2 UI/ml ADH (ADH_2).

presence of this detergent also was reflected in adenylate cyclase activity only in the subsequent stages of the hormonal effect.

To analyze the state of the intracellular stages of the response to ADH, after addition of Triton X-100 from the side of the basolateral membranes the response of the cells to cAMP was evaluated when cyclic nucleotide phosphodiesterase was blocked by theophylline. In the experiments with Triton X-100 the response to cAMP was depressed compared with experiments in which no detergent was added (Fig. 1). Since the ability of the cell to increase its permeability for water was reduced after the addition of forskolin and cAMP with theophylline, it follows that either the conditions for intercellular signal transmission were altered or (and this seems more probable) the ability of the apical membrane to be restructured was disturbed. Such a disturbance in the working of the cell could have taken place if the detergent exerted its influence not only on the basolateral membranes, but also by lateral diffusion of substances through the zone of the tight junction in the apical plasma membrane.

In connection with the facts described above, to assess the functional state of the cells of the mucus membrane of the bladder the detergent was added directly to the solution near the apical membrane of the cells. In this case ADH and forskolin were unable to increase permeability for water (Table 1). Consequently, the change in the state of hydrophobic interactions in the apical plasma membrane on the addition of detergent from the outer side of the membrane is of critical importance for ability to respond to ADH and for those structural changes in the membrane without which permeability for water cannot be increased.

It is well-known that the plasma membrane and the zone of intercellular junctions in epithelium with low permeability for water (skin and urinary bladder of amphibians, the terminal segment of the renal tubules) possess low permeability for ions. However, detergent may affect ionic permeability both of the plasma membrane and of the zone of intercellular junctions, and this would lead to a flow of potassium into the solution on the side of the bladder lumen from the cytoplasm of the cells and of sodium from the Ringer's solution in contact with the serous membrane. The results showed that in the low concentration used (0.1 mg/ml) Triton X-100 caused no marked increase in permeability of the apical plasma membrane for ions, and in many cases the sodium and potassium ion concentrations were close to their values in liquid taken from the lumen of bladders into whose lumen no Triton X-100 had been introduced (Table 1).

The low concentration of detergent used in these experiments and the relative specificity of its action, in the concentration used, on the effect of ADH on water metabolism, without any nonspecific increase in ionic permeability, suggests that this effect of Triton X-100 may be reversible. To investigate this possibility experiments were carried out in which 0.1 mg/ml of Triton X-100 was supplied to the serous membrane, and 30 min later the solution containing Triton was removed and replaced by fresh Ringer's solution with ADH or with cAMP and theophylline. In both cases an increase in the flow of water along the osmotic gradient was observed (Fig. 3). Thus in the concentrations used, the action of Triton X-100 was reversible in character.

These results are evidence of the important role of hydrophobic components of the membrane for ADH receptor and adenylate cyclase function. Exposure to the detergent even in a very low concentration abolishes the ability of the apical plasma membrane to react to ADH and to cAMP. Consequently, the hydrophobic component of plasma membranes play an important role in the realization of the cell's response to ADH. The possibility that the results of this investigation may be of applied importance must be mentioned. Under conditions of chronic contact with surface-active cationic agents [4], changes in diuresis and in excretion of ions by the kidney are found when detergents are introduced into the body [1]. One cause of renal pathology under the influence of these substances may be a change in stage of the membranes of the renal tubules, and their response to hormones and to other physiologically active substances.

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