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Surface Hydrophobicity and Water Transport of the Toad Urinary Bladder: Effects of Vasopressin

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Summary. The present study investigated whether the hydrophobic properties (wettability) of the luminal surface of the toad urinary bladder might play a role in modulating water transport across this epithelium. In the absence of vasopressin (ADH), water transport across the tissue was low, while luminal surface hydrophobicity (water contact angle) was relatively high. Following stimulation by ADH, water transport increased and surface hydrophobicity decreased. The addition of indomethacin to inhibit ADH-induced prostaglandin synthesis did not reduce these actions of ADH. In an attempt to alter water transport in this tissue, a liposomal suspension of surface-active phospholipids was administered to the luminal surface. This addition had no detectable influence on the low basal rates of water transport, but blocked the ADH-induced stimulation of water transport. We suggest that surface-active phospholipids on the toad bladder luminal membrane may contribute to the hydrophobic characteristics of this tissue. ADH may act to decrease surface hydrophobicity, facilitating the movement of water molecules across an otherwise impermeable epithelium. This surface alteration may be associated with the appearance of water channels in the apical membrane.

Key Words toad urinary bladder · surface hydrophobicity · vasopressin · phospholipids · water transport

Introduction

The luminal surface of the toad urinary bladder is relatively impermeable to water and solutes. However, in response to the peptide hormone vasopressin (ADH), its permeability to water markedly increases [2]. We questioned whether these permeability changes were related to the hydrophobicity of the luminal surface of the toad bladder epithelium as we have shown that the gastric epithelium, another tissue with a low permeability to water, has an exceedingly hydrophobic surface as assessed by contact angle measurement [9]. The contact angle refers to the angle between air, a fluid drop, and a surface and is directly proportional to hydrophobicity. In contrast to the stomach, the duodenum is highly water permeable and does not have a hydrophobic surface [9]. In addition gastric tissue can be protected from the damaging effects of excess hydrochloric acid by oral pretreatment of the stomach with a liposomal suspension of surface-active phospholipids [11, 13]. The purpose of the present study was to evaluate whether water transport and luminal surface hydrophobicity of the toad urinary bladder were related in ADH-stimulated tissue, and whether a luminal liposomal suspension of phospholipids would block the movement of water.

Materials and Methods

Water Transport

Paired urinary bladder sacs from the toad *Bufo marinus* were incubated at room temperature in frog Ringer's solution on the serosal side (111 mM NaCl, 3.4 mM KCl, 2.7 mM $CaCl_2$, 2.4 mM $NaHCO_3$, and 5.6 mM glucose) and a 1 : 4 dilution of this on the mucosal side. Arginine vasopressin (ADH, 20 mU/ml) was added to the serosal side. Water transport was monitored for 30 min before and after ADH treatment by the method of Bentley [2]. The change in weight of bladder sacs was monitored at 15-min intervals. To avoid possible effects from the accumulation of tissue prostaglandins, some experiments were performed after first incubating the bladders for 60 min in the presence of indomethacin (1 μM), a prostaglandin synthesis inhibitor.

In order to assess the effect of intraluminal phospholipid on ADH-induced water transport, either diluted Ringer's solution (1 : 4) or a liposomal suspension of surface-active phospholipid made in the same solution were incubated intraluminally in paired bladder sacs for 60 min. ADH (0.5 mU/ml) was then incubated serosally for 30 min and water transport was monitored. The phospholipid mixture consisted of 1.35 mg/ml dipalmitoyl phosphatidylcholine and 0.15 mg/ml phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol and sphingomyelin. The phospholipids were first dissolved in chloroform, evaporated under nitrogen, and then sonicated in the diluted Ringer's solution as described earlier [11]. Chemicals were obtained from

Table. Surface hydrophobicity and water transport in the toad urinary bladder

	Contact angle (degrees)	Water transport (mg H_2O/min/bladder)
Experiment 1		
Control	31 ± 4 (14)	0.9 ± 0.3 (14)
ADH	[a]23 ± 2 (14)	[a]19.4 ± 2.3 (14)
Experiment 2		
Control	39 ± 3 (14)	1.3 ± 0.4 (14)
Indomethacin	38 ± 0.4 (8)	1.2 ± 0.2 (8)
Indomethacin + ADH	[a]21 ± 6 (7)	[a]16.1 ± 3.2 (7)

Urinary bladder sacs were incubated for 60 min in the presence or absence of indomethacin (1 μM) and then for 30 min in the presence or absence of ADH (20 mU/ml). The change in weight of the tissue was used as an indication of water transport, and contact angle as measured on a goniometer was used to determine hydrophobicity. Values are expressed as the mean ± SEM for (n) determinations.
[a] $P < 0.05$ *vs.* control.

Sigma Chemical Co. (St. Louis, MO) and were of the highest purity available.

Surface Hydrophobicity

Hydrophobicity was estimated on a goniometer (Rame-Hart, Moutain Lakes, NJ) by measurement of the triple point contact angle, which formed between the air, the edge of a microdroplet of water (0.5 μl), and the luminal surface. This procedure has been used by our laboratory previously to study the hydrophobic surface properties of the dog gastrointestinal tract [9, 12]. Briefly, after incubation, the bladder was placed onto a wax board so that the luminal surface was exposed. The tissue was gently blotted with filter paper and allowed to air-dry for 15 min before readings were made. The drying time was necessary since contact angle readings could not be performed on a wet surface.

Tissue Viability

Experiments were performed to verify the viability of toad bladder, which had been exposed luminally to the phospholipid suspension. Sections of tissue were mounted in Ussing chambers and the voltage was clamped at zero using a Control Instruments, Inc. voltage clamp device. The resulting short circuit current was measured as an indicator of the net active transport of sodium. The bladder was exposed to the identical solutions and for the same time intervals as described previously for water transport studies.

Statistics

All values are expressed as the mean ± SEM. The paired Student's t test was used to calculate the difference between treatments with $P < 0.05$ as the level of significance.

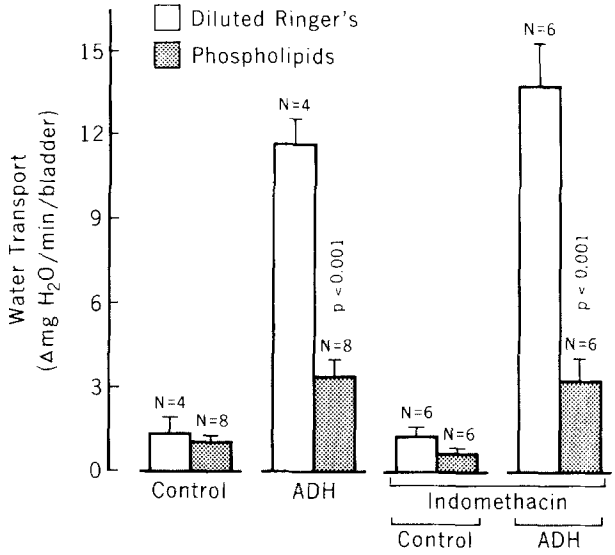

Fig. Phospholipids and water transport in ADH-stimulated toad urinary bladder. Urinary bladder sacs were incubated in frog Ringer's solution with an intraluminal addition (1.5 ml) of liposomal phospholipids made in diluted Ringer's solution. Following a 1-hr incubation with or without the intraluminal phospholipids and either in the presence or absence of indomethacin in the serosal bathing solution (10 μM), the bladders were exposed to a further control or ADH stimulation (0.5 mU/ml) for 30 min. Water transport was measured every 15 min. The values are expressed as the mean ± SEM

Results

As shown by the summary data in the Table, the contact angle of the toad bladder luminal surface was significantly decreased following ADH treatment. Concurrently, water transport by the bladder increased. When the experiment was repeated in the presence of indomethacin, results similar to those seen in the absence of the cyclo-oxygenase inhibitor were obtained.

The effect of the liposomal phospholipid mixture on water transport is summarized in the Figure. Normal ADH-induced water transport was blocked by the presence of intraluminal phospholipids (2.8 mM). Further, this phospholipid-induced inhibition of ADH-induced water transport did not appear to be mediated by an enhancement in tissue prostaglandin formation, as it was not affected by the addition of the cyclo-oxygenase inhibitor, indomethacin.

Finally, tissue viability remained high after exposure to luminal phospholipids. Short-circuit current was not significantly altered from control levels (23.4 ± 6.6 μA/cm^2, $n = 6$) by the addition of phos-

pholipid to the luminal incubation medium (107 ± 26% of control). Further, the phospholipid-treated tissue was able to respond to ADH, as 30 min after the hormone was added to the serosal side, the short-circuit current across the bladder had doubled (199 ± 53% of control, $P < 0.02$).

Discussion

We have interpreted these data to suggest that the hydrophobicity of the luminal surface of the toad urinary bladder is appreciable under resting conditions. This water-repellant characteristic is consistent with the fact that the bladder epithelium is relatively impermeable to water unless stimulated by ADH, at which time its surface is altered to allow the transport of water through water channels in the tissue. The consistent decrease in hydrophobicity following ADH stimulation described above may indicate that this event is a primary action of ADH on the toad bladder, occurring along with numerous other cellular alterations [1, 4–6, 10, 16].

The inclusion of indomethacin in our experimental procedure yielded data supportive of our contention that the effects we observed were due to a direct action of ADH and phospholipids on bladder water transport, rather than an indirect effect due to alterations in tissue prostaglandin accumulation [18]. Neither the decrease in surface hydrophobicity (Table), nor the phospholipid block of water transport (Figure) were affected when tissue prostaglandin synthesis was inhibited with indomethacin. These negative findings with indomethacin are of importance since it is well recognized that the synthesis of prostaglandins in the E series, which are known inhibitors of ADH-induced water transport in the bladder [15, 18], are increased by both phospholipid hydrolysis and ADH administration [18].

The mechanism of the ADH-induced reduction in luminal surface hydrophobicity is not known, but could be related to other actions of ADH. For example, the fusion of aggrephores with the granular cell apical membrane, which occurs following ADH stimulation [17], may be the initiator of the decrease in surface hydrophobicity. The particle aggregates (which may form water channels) could themselves be hydrophilic. Thus, their inclusion into the plasma membrane might dilute the hydrophobic character of the luminal surface to account for the ADH-induced transition to a more wettable surface. In a similar manner, increased intracellular water content in response to ADH could affect the hydrophobicity readings. Following blotting, this fluid could be pulled to the surface and form a wetter surface. Finally, the reduction in contact angle by ADH treatment could relate to the presence of mucus. The chief component of mucus (mucin) is a known wetting agent (7). Therefore, it is feasible that the presence of this substance on the luminal surface may account for a portion of the surface hydrophobicity changes described here. We are not able to distinguish between the above possibilities with our measurement of surface hydrophobicity. The contact angle would decline in response to a more wettable surface (mucus), a wetter surface (water), and a more permeable surface (the insertion of water channels).

We would suggest an alternative hypothesis for the ADH-induced decrease in luminal surface hydrophobicity based on our data obtained with phospholipids (Figure). We showed that exogenously added phospholipids blocked water transport induced by ADH. Amphoteric phospholipids phosphatidylcholine and phosphatidylethanolamine are known to readily adsorb to negatively charged surfaces resulting in an enhancement of their hydrophobic characteristics, and have been implicated in changes in the surface wettability of the lung and stomach [8]. Therefore, it can be postulated that surface-active phospholipids adsorbed to, or associated with, the luminal surface of the toad bladder may confer a hydrophobic, nonwettable barrier to the epithelium, possibly by binding to or blocking water channels. A number of investigators have reported that the rate of phospholipid hydrolysis is increased by ADH [3, 14, 18]. It is thus conceivable that ADH acts to increase the activity of enzymes, which alter surface-associated phospholipids, resulting in an increase in wettability (decrease in hydrophobicity) and the initiation of water transport through water channels of the bladder.

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