Effect of Distension on ADH-Induced Osmotic Water Flow in Toad Urinary Bladder

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Summary. We recently described a method by which the resistance to water flow of the luminal membrane of ADH-stimulated toad bladder can be quantitatively distinguished from that of barriers lying in series with it. This method requires estimates of both total bladder water permeability (assessed by transbladder osmotic water flow at constant gradient) and luminal membrane water permeability (assessed by quantitation of the frequency of ADH-induced luminal membrane particle aggregates). In the present study we examined the effect of bladder distension on transepithelial osmotic water flow before and during maximal ADH stimulation. Base-line water flow was unaffected by bladder distension, but hormonally stimulated flow increased systematically as bladders became more distended. Distension had no effect on the frequency of ADH-induced intramembranous particle aggregates. By comparing the relationships between aggregate frequency and hormonally induced water permeability in distended and undistended bladders, we found that distension appeared to enhance ADH-stimulated water flow by decreasing the resistance of the series permeability barrier while the apparent water permeability associated with each single luminal membrane aggregate was unaffected. In that bladder distension causes tissue thinning, the series resistance limiting ADH-stimulated water flow appears to be accounted for by deformable barriers within the bladder tissue itself, probably unstirred layers of water.

Key words antidiuretic hormone · toad urinary bladder · tissue distension · osmotic water permeability

Introduction

The isolated toad urinary bladder has been extensively used to study the mechanism of action of antidiuretic hormone (ADH). ADH acts upon the epithelial cells which line the bladder, increasing their luminal membrane permeability to water and small solutes, most notably sodium and urea. It seems clear that ADH-stimulated water movement occurs through pore-like pathways [10, 12, 17, 26] which are selective for water alone [21, 23, 24, 29] and restricted to granular-type cells only [6, 7, 28]. Freeze-fracture electron microscopy reveals that ADH also causes the occurrence of intramembranous particle aggregates in granular cell luminal membrane [3, 19]. These aggregates are derived preformed [5, 14] from membranes of long, cytoplasmic tubular structures [13, 30] which fuse with the luminal membrane consequent to ADH treatment [27, 31]. The hypothesis that aggregates may be or may contain actual sites for ADH-stimulated water flow across the luminal membrane has not yet been directly proved. Nonetheless, there is considerable evidence which indicates that, as a minimum, aggregates are accurate markers of hormonally stimulated luminal membrane water permeability [2, 4, 8, 9, 14–20, 23].

Because of the profound effect of ADH on luminal membrane water permeability, there has been a tendency to underestimate the importance of barriers to transbladder flow other than the luminal membrane. In fact, in our original studies in which 17 fully distended bladders were stimulated with various concentrations of ADH, the relationship between hormonally enhanced osmotic water permeability and luminal membrane aggregates appeared to be linear [19]. We recently re-examined this question in a larger group of fully distended, ADH-stimulated bladders [22]. We found that the relationship between aggregates and osmotic water permeability was generally similar to that which we had observed earlier; however, it became apparent that the linear regression line relating aggregates and induced water permeability consistently underestimated water permeability at both low aggregate frequency (i.e., it did not pass through the origin) and high aggregate frequency, while it tended to underestimate water permeability at intermediate aggregate frequency. A more detailed analysis of these data revealed that the data closely approximated the "saturation curve" relationship expected if aggregate frequency were proportional to luminal membrane water permeability while a (constant) flow barrier in series with the luminal membrane became increasingly limiting for transbladder water movement as luminal permeability approached a maximum.
Based upon this formulation for barriers in series, we calculated that in fully distended bladders, maximally stimulated with ADH, this series barrier contributes between one-half and two-thirds of the total transbladder resistance to water flow [22]. Our data did not, however, permit us to localize the series barrier within the bladder tissue. The present report includes an approach to this issue, based upon alterations in series barrier resistance that accompany different states of bladder distension.

The toad bladder is an easily distensible organ. Gfeller and Walser have studied toad bladder morphology in relation to different filling volumes [11]. They demonstrated that the bladder's luminal surface area remains virtually constant with changes in filling volume. At maximal filling volume, the luminal membrane is practically a flat sheet with some microvilli. As filling volume decreases, the luminal membrane becomes undulating and microvilli become both more numerous and more prominent. In addition, as bladder filling approaches maximum, epithelial cells and supporting tissues are distended so that the luminal and serosal bathing media are separated by a smaller thickness of tissue.

Walser has previously demonstrated that bladder distension increases transbladder sodium movement [32]. This effect is reversible and appears to involve only the conductance of the active sodium transport pathway [25, 32]. In contrast, transepithelial urea movement both in the absence and presence of ADH has been shown by Lief et al. to be unaffected by bladder distension [25].

In the present investigation we measured the effect of bladder distension on transbladder water movement before and during bladder stimulation with both maximally and submaximally stimulating concentrations of ADH. We found that while the occurrence of aggregates in granular cell luminal membrane was unchanged, “maximal” ADH-stimulated transbladder osmotic water flow was systematically enhanced as bladder distension increased. Comparison of the relationships between aggregate frequency and induced osmotic water permeability in undistended and distended bladders at various concentrations of ADH suggests that distension increased osmotic water flow by decreasing the resistance of the series permeability barrier while the luminal membrane water permeability associated with each aggregate was not appreciably altered. In view of the morphologic effect of bladder filling, which we confirmed, our data are consistent with the view that the series barrier resistance limiting ADH-stimulated water movement in toad bladder is largely, if not totally, accounted for by tissue unstirred layers of water.

### Materials and Methods

In the first set of experiments in this study the effect of various degrees of bladder filling on ADH-stimulated osmotic water flow was examined. Paired bladders from 16 female Dominican toads (Bufo marinus) were mounted as sacs on the ends of glass tubes, suspended in vigorously aerated Ringer solution (111 mM NaCl, 3.5 mM KCl, 2.5 mM NaHCO₃, 1.0 mM CaCl₂, 220 mosmol/Kg H₂O), and filled either to total capacity1 or to a lesser extent with Ringer's diluted 1:5 with distilled water. For all bladders, filling was always sufficient to bathe the entire mucosal surface. In an additional series of experiments, paired bladders from six other toads were prepared in the same manner, except that the mucosal bathing medium of the submaximally distended tissues was stirred by a suspended, rotating magnet.

After a 30-min equilibration period, transbladder open-circuit potential was measured, and if less than 20 mV, the experiment was terminated. Otherwise, water movement was determined gravimetrically [1] first for a 30-min base-line period and then for a 20-min period in the presence of ADH (Pfressin, Parke-Davis, Detroit, Mich.) at a concentration (20 mU/ml serosal solution) sufficient to induce a maximal hydro-osmotic response. After the final water flow measurement, each submaximally filled bladder was maximally filled with a measured volume of fluid and bladder volume capacity was calculated. All bladders were then blotted gently to remove adherent fluid, and weighed. The total luminal surface area of every bladder was calculated from its maximal volume capacity on the assumption that the lumen of a fully distended bladder sac is a smooth sphere [11]. This surface area estimate and wet tissue weight were both used to normalize water flow for the various bladders studied here.

In all sac preparations studied a mucosal hydrostatic pressure of approximately 2–4 cm H₂O was always present. Bladders that were maximally filled, tended to have higher mucosal hydrostatic pressures than those which were submaximally filled. In validation experiments with fully distended, paired bladders (n = 6), we found no measurable effect of a difference in hydrostatic pressure between 2.4 ± 0.2 and 5.0 ± 0.2 cm H₂O on either base-line or ADH-stimulated transbladder osmotic water movement.

In a second set of experiments, morphologic effects of distension were examined by thin-section electron microscopy in bladder pairs unexposed (n = 2) and exposed to ADH (n = 6). In addition, for the bladders exposed to hormone, the frequency of induced intramembranous particle aggregates was assessed in freeze-fracture replicas. The procedure in these experiments was generally similar to that described above. Since the intent of these experiments was to examine morphologic aspects of bladder distension, however, the submaximally distended bladders (which were filled approximately between 40 and 60% of capacity) were not fully distended at the end of an experiment. Instead, both they and the maximally distended paired tissues, while still secured to glass tubes, were fixed with an isotonic solution of buffered glutaraldehyde (1.3% in 0.05 M cacodylate buffer at pH 7.4) from the serosal side for 1 min to preserve microscopic shape, then drained and cut from their tubes, and replaced in the same fixative for 30 additional min. The morphologic methods which followed thereafter are described elsewhere [15, 19].

In the final experimental set, 24 bladders from 14 toads were filled to 68 ± 1% of volume capacity (as determined at the end

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1 Bladders were considered to be filled to capacity and fully distended when the continued addition of solution to the preparation resulted only in increasing the volume contained in the cannula with no further increase in the volume of the bladder. Bladder volume capacity was calculated by subtracting the volume of solution in the cannula from the total volume added to the preparation.