PHYSIOLOGY

Hyaluronidase Increases the Osmotic Resistance of Frog Urinary Bladder

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Hyaluronidase (30 U/ml) added from the mucosal side of the frog urinary bladder (pH 5.4, 25°C) is shown to increase the permeability of its wall for water and to shorten fibrillary structures of the apical glycocalyx. The hyaluronidase-mediated increase in osmotic permeability is smaller, and occurs later, than that produced by arginine vasopressin.

Key Words: *vasopressin; hyaluronidase; water permeability*

Some 40 years ago, in an article published in the *Bulletin of Experimental Biology and Medicine*, Ginetsinskii and coauthors reported that hyaluronidase is released by the kidneys [1]. Four years later, Ginetsinskii advanced a hypothesis attributing a role to this enzyme in the mechanism of action of antidiuretic hormone (ADH) [7]. This hypothesis has not gained wide acceptance, in particular because there is no direct evidence to indicate that osmotic resistance is increased by added hyaluronidase. Previous attempts to increase water permeability by adding hyaluronidase from the mucosal side of the amphibian urinary bladder - the main physiological model used to study the mechanism of action of ADH - have been unsuccessful [5,10]. In the present study, an attempt was made to use a new methodological approach to test hyaluronidase for its effect on the osmotic resistance of the amphibian (frog) bladder wall.

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MATERIALS AND METHODS

The study was conducted on urinary bladders from winter *Rana temporaria* frogs. Hyaluronidase was added from the mucosal side of the bladder while the serosal side was bathed with Ringer's solution. The measure of bladder wall permeability was a water flow via an osmotic gradient (expressed in μl/min× ×cm^2). One portion of the bladder served as the control while the other portion was exposed to hyaluronidase (EC 3.2.1.35; Type I-S), 300 U/mg; Sigma) or to arginine vasopressin (Serva). The method used to assess water permeability was similar to that described previously [6] except that the two bladder portions were weighed on an electronic balance (Gosmetr, St. Petersburg) and the results automatically entered into a computer.

For electron microscopic examination, each portion of the bladders was placed for 5-10 min in a 2.5% isotonic glutaraldehyde solution in phosphate buffer (pH 7.4), after which the bladders were cut, fixed in the same buffer for 60 min, washed in a buffered isotonic sucrose solution, and transferred to a 1% OsO_4 solution for 60 min, followed by dehydration by asending alcohols in absolute acetone, em-

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bedding in Epon-Araldite, and preparation of ultrathin sections. These were contrasted with lead citrate and uranyl acetate and examined in a JEM-100U electron microscope.

RESULTS

After 30 U/ml of hyaluronidase were added to the hypotonic Ringer's solution at the mucous membrane or to Ringer's solution at the serous membrane, no change in water permeability was recorded, just as in previous studies where no changes were detected in the permeability of toad [10] or frog [5] urinary bladders for water. Analysis of our results led us to suppose that the experimental conditions used did not take into account the optimum pH (5.4) for hyaluronidase activity: the enzyme had been isolated from mammalian tissues rather than from tissues of coldblooded animals, and the temperature factor should therefore have been allowed for. Accordingly, a 0.02 M acetate buffer was prepared (pH 5.4, osmolarity 30 mOsm/kg H_2O) to which hyaluronidase was added and the tests were run in thermostatically controlled vessels at 25°C. The hyaluronidase added from the mucosal side was found to have increased the water permeability by up to 604% relative to the baseline level (Fig. 1, curve *2*)). The acetate buffer without hyaluronidase did not affect the epithelial permeability for water (Fig. 1, control). Of the hyaluronidase concentrations tested (15 to 300 U/ml), 30

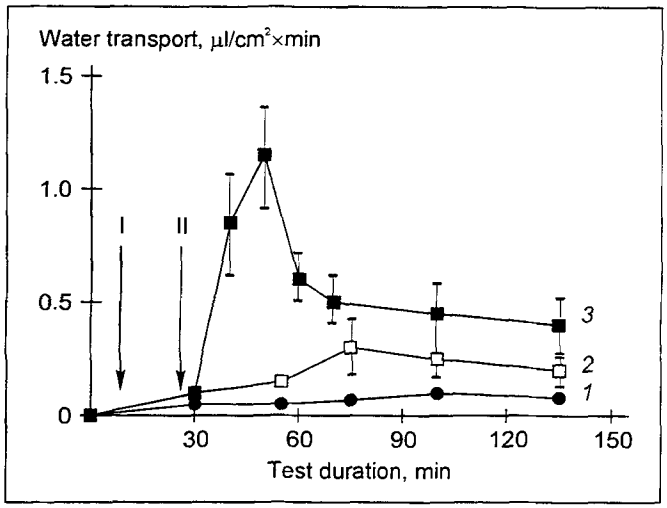

Fig. 1. Effects of arginine vasopressin (1.7×10^{-8} M) and hyaluronidase (30 U/ml) on water permeability of frog bladder wall. Arrows: *I*) hyaluronidase added to Ringer's solution at the mucous membrane (curve *2*; n=9); *II*) arginine vasopressin added to Ringer's solution at the serous membrane (curve *3*; n=15); curve *1*) control tests (n=8).

U/ml proved the most effective one, higher concentrations failing to increase the enzyme's effect on water permeability. When added from the serosal side, the enzyme did not alter water permeability. It should be noted that although the Ringer's solution had a temperature of 25°C, hyaluronidase was added without acidifying the solution with acetate buffer because under natural conditions the pH of urine may fall to below 5.0 and the apical membranes of

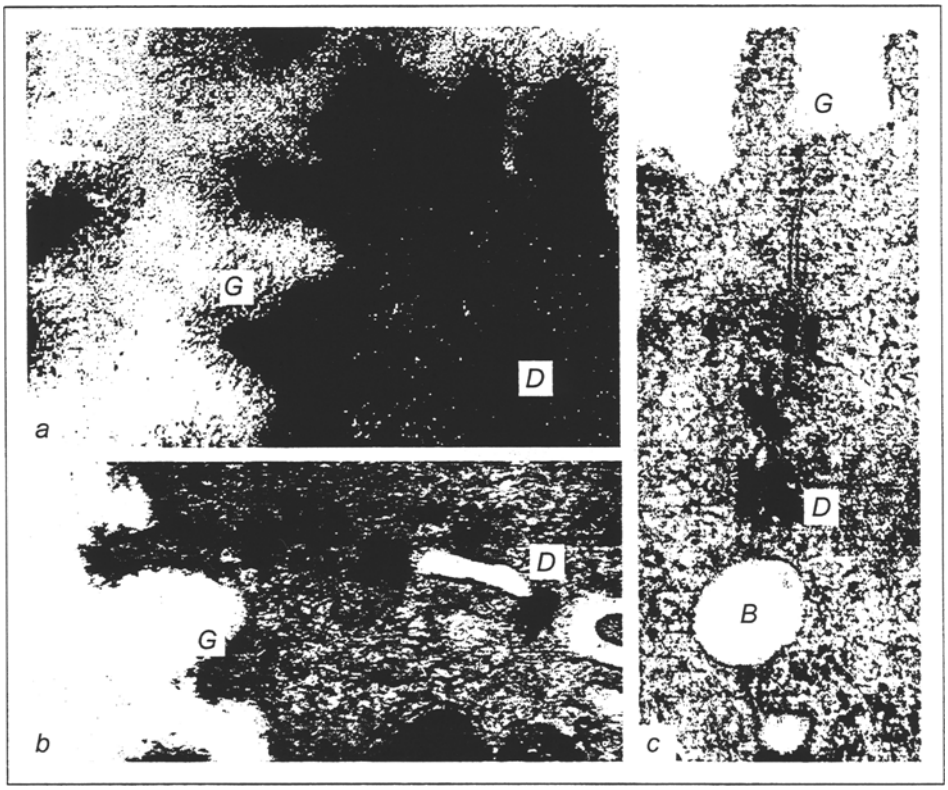

Fig. 2. Effects of hyaluronidase on ultrastructure of granular cells in frog bladder. *a*) control sample; *b* and *c*) hyaluronidase-treated samples. *G* = glycocalyx; *D* = desmosome; *B* = blister.

cells are therefore adapted to function at acid pH's. Basolateral membranes always function at the pH's characteristic of blood and extracellular fluids and a fall of the pH to below 7.0 renders the cells incapable of responding to ADH. The greatest increase in water permeability (by 2950%) occurred 15-20 min after the addition of 1.7×10^{-8} M vasopressin (Fig. 1, curve *3*).

The ultrastructure of granular cells in the intact bladder epithelium is characterized by a prominent finely fibrillated glycocalyx on the surface of microvilli and in free areas of the apical membrane [3] (Fig. 2, *a*); in the region of cell attachment, specialized cell junctions (tight junction, intermediate junction, and desmosomes) can be seen. After hyaluronidase was added to the solution at the mucous membrane, the cytoplasmic structure remained unchanged, whereas fibrils of the glycocalyx lost their fine structure and became shorter (Fig. 2, *b*). The tight junction had the same structure as before, but blisters or widened intercellular clefts between desmosomes were noted in some cases (Fig. 2, *c*). The structure of the basal portions of the cells remained unchanged.

Hyaluronidase caused a much smaller increase in water permeability than did vasopressin. It has been shown that the increase in osmotic permeability elicited by vasopressin is due to the activation of V_2 receptors and the formation of cAMP [5] and to the incorporation of water channels into the luminal membrane of the cell [4,8]. When we look at our results and compare them with those reported by other authors, we may conclude that the hyaluronidase mechanism serves as a further means for increasing water permeability, although the increase is not additive to that caused by vasopressin.

The action of hyaluronidase from the external mucosal surface is consistent with the original ideas of Ginetsinskii that hyaluronidase secretion by epithelial cells of the nephron is of the apocrine type and that the enzyme acts from the luminal side of the tubule [7]. Undoubtedly of prime importance in increasing the water permeability of the apical membrane are water channels [8], whose incorporation is stimulated by ADH (and cAMP) [11]. Hyaluronidase probably depolymerizes glycosaminoglycans on the outer surface of the luminal membrane, a process which is reflected in an altered structure of the glycocalyx and which sets the stage for a small increase in water flow by pathways of osmotic permeability. The observed small widening of the intercellular area beneath the cell junctions supports this possibility. Hyaluronidase is secreted not only into the canalicular lumen but also into the pericanalicular fluid [2,9]; the depolymerization of glycosaminoglycans from the serosal side by hyaluronidase could be of some importance for improved outflow of reabsorbed water. It is possible that under natural conditions lysosomal hydrolases undergo exocytosis with local acidification of the medium and subsequent hydrolysis or else several enzymes are released to hydrolyze glycosaminoglycans consecutively. In the epithelium of osmoregulatory organs (collecting tubules of the kidney, amphibian urinary bladder), the role of hyaluronate hydrolases may be presumed to consist in facilitating water movement, while the main mechanism regulating water permeability in response to ADH is provided by water channels whose incorporation into the luminal membrane is mediated by vasopressin.

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