The Role of Water Diffusion in the Action of Vasopressin

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Summary. Vasopressin produces a large increase in the osmotic flow of water across the toad bladder, with little apparent change in the diffusion rate of tritiated water. This discrepancy between osmotic and diffusional net flow is the basis of the pore theory of vasopressin action. The present studies show that there is in fact a large (at least 10-fold) increase in water diffusion subsequent to addition of vasopressin, which is masked by unstirred layers and by the resistance offered to diffusion by the thick layer of connective tissue and muscle supporting the bladder epithelial cells. An even higher diffusion rate would be anticipated with the complete elimination of unstirred layers, and of barriers to diffusion remaining within the epithelial layer itself. An alternative to the pore hypothesis is considered, in which vasopressin acts solely by increasing the diffusion rate of water across the luminal membrane of the epithelial cell.

It is generally believed that the osmotic flow of water across living cells is the result of Poiseuille flow through aqueous channels (pores) in the cell membrane [14, 19]. This view is based on the observation that the rate of diffusion of labelled water across cells does not appear to be high enough to account for the large water flows observed. In the isolated urinary bladder of the toad, for example, total osmotic flow exceeds the net flow predicted from the rate of diffusion of tritiated water (THO) by a ratio of 6:1. When the bladder is treated with vasopressin, osmotic flow increases 40-fold, with only a small apparent increase in diffusion, and the ratio of osmotic to diffusional net flow becomes greater than 100:1 [11].

It is possible to arrive at an estimate of the mean radius of the pores required to produce such a discrepancy between osmotic and diffusional net flow by employing the following expressions. For osmotic flow,

$$\frac{L_p}{\nabla_w} = \frac{n \pi r^4 \Delta P}{8 \eta \Delta x};$$

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for net diffusional flow,

$$\omega_T = \frac{n \pi r^2 D \Delta P}{RT \Delta x},$$  \hspace{1cm} (2)

where $L_p$ is the hydraulic water flow in the presence of a hydrostatic or osmotic driving force, $V_w$ the molar volume of water, $n$ the number of pores, $r$ the mean pore radius, $\Delta P$ the gradient of pressure, $\eta$ the bulk viscosity coefficient of water, $\Delta x$ the membrane thickness, $\omega_T$ the net diffusional flow, $D$ the self-diffusion coefficient of water in water, $R$ the gas constant and $T$ the absolute temperature.

When the terms are combined, common terms cancel out, yielding the expression

$$\frac{L_p}{V_w} \omega_T = \frac{r^2 RT}{8\eta V_w D}.$$  \hspace{1cm} (3)

Therefore, if $L_p/V_w$ and $\omega_T$ are determined experimentally, and expressed as a ratio, the mean pore radius can be estimated. The high apparent ratios in the case of the toad bladder yield values for pore radius of 8 Å in the absence of vasopressin and approximately 40 Å after exposure to the hormone.

This report presents evidence that the rate of diffusion of water across the vasopressin-treated toad bladder (and therefore $\omega_T$) has been greatly underestimated, and that the process of diffusion may play an important role in the action of the hormone. Unstirred layers of water in apposition to the bladder, and the thick layer of muscle and connective tissue supporting the bladder epithelial cells have been found to contribute significantly to the resistance of the bladder to diffusion; when the contribution of these layers is taken into account, the true rate of diffusion of water across the epithelial cells is found to rise strikingly after vasopressin is added.

**Materials and Methods**

Bladder halves were removed from doubly pithed female Dominican Republic toads (*Bufo marinus*) supplied by National Reagents Inc. (Bridgeport, Conn.). Bladder halves were mounted in lucite chambers with 50 ml phosphate-buffered Ringer's solution bathing each side. The Ringer's solution contained: 120 mM Na+, 4.0 mM K+, 0.5 mM Ca++, 116 mM Cl−, 1.0 mM H2PO4−, 4.0 mM HPO42−; pH 7.4; 230 mosm/kg H2O. A nylon hair net was stretched across each side of the bladder to hold it rigidly in position during stirring. Stirring was provided by four-bladed paddles cut from Teflon homogenizers; the paddles were attached to Cenco variable-speed stirrers (Central Scientific Co. Mountainside, N.J.), and entered the chambers through holes in the top (Fig. 1). The temperature of the bathing solutions was not raised by the stirring, and therefore was not a factor in the diffusion and flow rates obtained.