Effect of vasopressin and cyclic AMP on permeability of isolated collecting tubules

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GRANTHAM, JARED J., AND MAURICE B. BURG. Effect of vasopressin and cyclic AMP on permeability of isolated collecting tubules. Am. J. Physiol. 211(1): 255-259. 1966.—The effect of vasopressin and cyclic 3',5'-AMP on the permeability to water and urea of the isolated perfused rabbit collecting tubule was measured. When added to the solution bathing the outside of the tube, these agents regularly increased both net water absorption along an osmotic gradient and the diffusional permeability to water as measured with THO in the absence of an osmotic gradient. When added only to the solution bathing the luminal border of the cells, vasopressin was ineffective. Urea permeability was not significantly altered by either vasopressin or cyclic 3',5'-AMP despite simultaneous increases in water permeability.

Knowledge of the mechanism of action of antidiuretic hormone derives largely from studies of anuran membranes. Vasopressin increases both the diffusional and osmotic permeability to water of these epithelial structures. These effects presumably result from enlargement of aqueous channels or pores within the membranes (7, 11). In some, the permeability of the membrane to urea is also enhanced by vasopressin (13). The hormone is effective only when applied to the serosal surface of the membrane (13) and its action involves increased production of cyclic 3',5'-AMP which in itself has been shown to duplicate the effects of vasopressin (6, 16).

It has been assumed that vasopressin has a similar effect on the mammalian collecting tubule, although direct proof has been difficult to obtain owing to the relative inaccessibility of this structure in vivo. From studies of renal function there is evidence that the osmotic permeability of the collecting tubule to water is enhanced by antidiuretic hormone (19), and it has been suggested that the increased absorption of urea which has also been observed under these circumstances is due to an increase of urea permeability (2, 8, 9).

In vitro microperfusion of isolated renal tubules affords a means to study otherwise inaccessible portions of the nephron such as the collecting tubule in a direct and controlled fashion (4). In the present studies the effect of vasopressin and cyclic 3',5'-adenosine monophosphate (cyclic 3',5'-AMP) on water and urea absorption by collecting tubules dissected from cortex and outer medulla of the rabbit has been measured. Both agents caused an increase in diffusional and osmotic water permeability without significantly altering the permeability to urea. As in anuran membranes vasopressin was effective only when applied to the peritubular or blood border of the tubule cell.

METHODS

Fragments of collecting tubules from kidneys of young New Zealand white rabbits were dissected and perfused using methods previously described (4). The outside bathing medium used for dissection and perfusion contained NaCl 115 mM, KC1 5 mM, NaHCO3 25 mM, Na acetate 10 mM, NaH2PO4 1.2 mM, MgSO4 1.2 mM, CaCl2 1.0 mM, 5% v/v calf serum (Microbiological Associates) and dextrose 5.5 mM. (Osmolality of this solution was 290 milliosmoles kg⁻¹.) Two perfusion solutions were used. 1) "isotonic" perfusion solution (290 milliosmosols kg⁻¹) containing NaCl 150 mM, K2HPO4 2.5 mM, MgSO4 1.2 mM, and CaCl2 1.0 mM, pH adjusted to 7.4 with dilute HCl; and 2) "hypotonic" perfusion solution (70 milliosmosols kg⁻¹) identical to the isotonic solution except that the concentration of NaCl was 30 mM. Tubules 0.7-2.7 mm long were transferred to a chamber containing approximately 2.5 ml of the outside bathing solution through which the gas mixture (95% O2, 5% CO2) was bubbled. Collections were generally started within 30 min after removal of the kidney from the rabbit. THO (final concentration 1 mc ml⁻¹) and/or urea C14 (New England Nuclear; 5.6 mc ml⁻¹, final concentration 1 μCi ml⁻¹) were added to the perfusion fluid in some experiments to measure water and urea permeabilities. Perfusion rate was constant during each experiment at from 13.3 to 20.0 ml min⁻¹. During perfusion the internal diameter of the tubules was 20 ± 3 μ (mean ± SD) and the outside diameter was 37 ± 5 μ. Accumulated fluid was removed from the collecting pipet...


TABLE I. Effect of vasopressin and cyclic 3',5'-AMP on water absorption from isolated perfused collecting tubules

<table>
<thead>
<tr>
<th></th>
<th>Control*</th>
<th>Change With Vasopressin,*</th>
<th>Change With Cyclic 3',5'-AMP*, 10^{-2} M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Isotonic perfusion</td>
<td>Hypotonic perfusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Net water absorption</td>
<td>1.53±.36 (11)</td>
<td>+3.37±.57 (10)</td>
<td>+2.15 (1)</td>
</tr>
<tr>
<td>Unidirectional water efflux</td>
<td>22.4±3.7 (6)</td>
<td>+32.6±5.0 (5)</td>
<td>+17.6 (1)</td>
</tr>
</tbody>
</table>

* μlitters cm⁻² min⁻¹ ± SEM for given number of experiments.

Net water absorption was determined gravimetrically at 10- to 20-min intervals with a calibrated glass capillary (54.1 ± 1.2 μ (sn) i.d.) and the length of the fluid column in the capillary measured to determine the volume of the collection. Usually a single capillary was used in each experiment for measuring both the standard solutions and all samples of collected fluid, thus reducing the variability of the measurements of volume to below 3%. Samples were washed out of the capillaries with scintillation fluid into 5 ml liquid-scintillation solution (4) and counted using a Packard Tri-Carb liquid-scintillation spectrometer. All experiments were performed at room temperature (23–24°C).

During perfusion the outside bathing solution was frequently replaced. After at least two consecutive stable control periods, a test substance was added to the outside bath and collections continued until a new steady state was reached. Vasopressin (Pitressin; Parke, Davis) was diluted to a final concentration of 25 or 250 μU ml⁻¹ with the outside bath solution immediately before use. (In experiments in which vasopressin was included in the perfusion solution and added later to the outside bathing solution both solutions were made up from the same ampoule of Pitressin prior to the beginning of the experiment.) In a control experiment the preservative in Pitressin (chlorobutanol) had no effect on net water absorption during perfusion of hypotonic solution when added to the outside bathing solution in the same concentration as is present in 25 μU ml⁻¹ Pitressin. The nucleotides, cyclic 3',5'-AMP and 5'-AMP (Calbiochem) always were added to the outside bathing solution in a concentration of 10⁻² M and neutralized with NaOH.

When there is no net fluid absorption, unidirectional THO or urea C¹⁴ permeability (in the outward direction) can be calculated as:

\[ \text{unidirectional permeability} = \frac{V_i}{A} \ln \frac{C_i}{C} \]  

where \( C_i \) is the concentration of C¹⁴ or THO in the perfused fluid, \( C \) is the concentration of C¹⁴ or THO in the collected perfusion fluid, \( V_i \) is the rate of perfusion (μlitters min⁻¹), and \( A \) is the luminal surface area (cm², calculated from the length of the tubule used, assuming a diameter of 20 μ). When there is net fluid absorption it has been assumed that the change in flow velocity is linear with respect to tubule length. Then,

\[ \text{unidirectional permeability} = \frac{V_i - V_s}{A} \ln \frac{C_i/C}{V_i/V_s} + 1 \]

where \( V_s \) is the collection rate. For net fluid absorption not exceeding 25% the assumption of linear absorption does not lead to a result which is significantly different from that calculated on the assumption that the rate of absorption is proportional to the difference in osmolality across the tubule cells, and that there is no loss of osmotically active solute from the lumen. Since the outside bath contained more than 2 ml of solution and the perfusion rate was less than 20 nl min⁻¹, there was no significant accumulation of radioactive isotope in the outside bath between changes of the bath fluid.

In general, unidirectional water permeability (expressed as litters cm⁻² min⁻¹) has been labeled “water efflux” to avoid implications concerning the mechanism of water permeation. However, in those studies in which there is no osmotic gradient and net water movement is small it is considered that the principal mechanism of water and urea permeability is diffusion. In order to emphasize this and facilitate comparison with other similar studies, the calculated “diffusional” water and urea permeabilities are listed using units of centimeters second⁻¹ in the appropriate tables and figures.
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RESULTS

Most collecting tubules were perfused for 6-8 hr and during this time the tubule cells were examined frequently at 100-400 X magnification. The appearance of the cells generally did not change. In preliminary experiments a perfusion solution identical to the outside bath had been used. After approximately 2 hr the tubule cells gradually desquamated, leaving denuded areas on the basement membrane. As this progressed, water and urea permeability increased and eventually collections ceased since all the perfused fluid leaked through the bare basement membrane. In contrast, desquamation occurred only rarely when the perfusion fluids described above were used. The experiments in which it did occur have been discarded. The reason for the differing effects of these solutions is not known.

The earliest collections from each perfused tubule are not included in the calculation of the results shown in the tables since diffusional and osmotic permeability to water regularly decreased initially (Figs. 1-4). A steady state generally was achieved within 220 min and the control values shown were calculated for each tubule from the mean of 2-4 collections during the steady state. It is not known whether factors other than the presence of endogenous antidiuretic hormone were responsible for the initially high water permeability.

Net fluid absorption in the control periods was greater in the presence of an osmotic gradient than when the luminal perfusion solution was isotonic (Table 1). Unidirectional water efflux greatly exceeded net water absorption and the former did not change significantly when an osmotic gradient was added (Table 1). Vasopressin regularly increased the permeability of the tubule to water, both as determined by net absorption along an osmotic gradient and THO efflux (Table 1). Similarly, urea permeability was not elevated at the beginning of the experiments as was water permeability (Figs. 3, 4).

In two experiments vasopressin was ineffective when added to the luminal perfusion fluid. One of these is illustrated in Fig. 4. In this experiment the control permeability to water (29 \( \times \) 10^{-9} cm sec^{-1} \( \times \)) was approximately the same as in the absence of the hormone (Table 2) despite the presence of 250 \( \mu \)U ml^{-1} vasopressin in the luminal fluid. Addition of 25 \( \mu \)U ml^{-1} vasopressin to the outside bath had the usual effect on water permeability (without any effect on urea permeability), showing that the tubule is capable of responding to vasopressin normally, provided that hormone is present at the contraluminal border of the cells.

DISCUSSION

Toad bladder and toad and frog skin have long served as models for detailed study of the mode of action of anti-

1 Occasionally, large clear vacuoles appeared in a few cells. The significance of the vacuoles is unknown, but there were no changes in experimental results coincident with their appearance.

FIG. 2. Effect of cyclic \( 3', 5' \)-AMP and vasopressin on unidirectional THO permeability. Perfusion solution was isotonic. Note that permeability reached a steady state after 120 min of perfusion.

FIG. 3. Effect of \( 5' \)-AMP and \( 3', 5' \)-AMP on unidirectional THO and urea-C\( ^4 \) permeability of collecting tubule. Perfusion solution isotonic.
diuretic hormone. Although it has been apparent that the mammalian distal nephron responds to vasopressin in a qualitatively similar manner, direct quantitative studies have been lacking. Recent micropuncture studies of rat distal tubule (5, 12, 18) and the present data now provide direct information concerning the permeability characteristics of these structures.

Net water flux in the absence of antidiuretic hormone is small in toad bladder and frog skin compared to rabbit collecting tubule and rat distal tubule (Table 3). Since the measurements in these tissues were performed at different temperatures and under a variety of conditions, and since only plane surface area, not the area of the sensitive membrane, was estimated in each case, it is difficult to be certain whether the absolute differences have any special significance. However, the relatively large net water flux in the mammalian distal nephron in the absence of antidiuretic hormone is consistent with previous observations of significant distal water absorption in the dog during water diuresis (3).

Diffusional water permeability measured with tracers greatly exceeds net water flux along an osmotic gradient in each of these tissues. However, it has been shown that diffusion of water can account for only a small fraction of the net water flux along an osmotic gradient through anuran membranes (7). In the collecting tubule, using the same calculation, only 3/5 of the net water flux along an osmotic gradient can be accounted for by diffusion. Thus, as in frog skin and toad bladder, net water flux through the collecting tubule in the absence of vasopressin is largely nondiffusional and probably due to bulk flow of water through aqueous channels or "pores."

Addition of antidiuretic hormone to toad bladder increases the fraction of the total net water flow that is nondiffusional (7). This is the change expected if the hormone increases the radius of pores or aqueous channels in the membrane. In the collecting tubule, as well, antidiuretic hormone increases nondiffusional water flow; however, in contrast to other structures studied, a large increase in the diffusional component is also observed so that the fraction of total net flow that is nondiffusional is essentially unchanged. Thus, it appears that vasopressin has a relatively greater effect on total pore area, as compared to mean pore radius, in the collecting tubule than in anuran membranes.

Vasopressin increases the permeability to urea of the toad bladder (15) but not of rat distal tubule (5) or rabbit collecting tubule. The failure to detect an increase in urea permeability is somewhat surprising, particularly since clearance studies in the dog (2, 8, 9) have been interpreted as indicating that the hormone increases urea absorption in the collecting ducts. A possible explanation for this discrepancy is that the segment of the collecting tubule in inner medulla does respond to vasopressin with increased urea permeability, whereas the portion in cortex and outer medulla, which was studied here and which differs histologically (20), does not. This interpretation is consistent with the findings of Kil and Aukland (2, 10) in stop-flow studies. An additional possibility is that the increased urea absorption observed in vivo is due to the greater urea concentration in the lumen during antiuresis rather than to an increase in urea permeability. Recently, it has been proposed that there are independent effects of vasopressin on urea and water permeability in toad bladder and that these effects occur at different permeability barriers in the membrane (14). The present finding that vasopressin increases water permeability without altering urea permeability is consistent with the view that two separate mechanisms are involved and that the permeability barrier to urea in collecting tubules has little if any sensitivity to vasopressin. The findings are also consistent with the possibility that water and urea may permeate these membranes through different channels. This possibility can be tested experimentally by determining whether "solvent drag" for urea exists under these circumstances.

Anuran membranes are sensitive to vasopressin when the hormone is presented to their blood (serosal) surface but not when it is presented to their epithelial surface.
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TABLE 3. Comparative permeability of several vasopressin-sensitive structures

<table>
<thead>
<tr>
<th>Antidiuretic Hormone</th>
<th>Toad Bladder (7, 15)</th>
<th>Toad Skin (11)</th>
<th>Distal Tubule In Vivo (3, 12, 18)</th>
<th>Collecting Tubule In Vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>.2</td>
<td>.29*</td>
<td>8.3*</td>
<td>2.8</td>
</tr>
<tr>
<td>Present</td>
<td>7.6</td>
<td>.66*</td>
<td>17.2*</td>
<td>9.1</td>
</tr>
<tr>
<td>None</td>
<td>9.4</td>
<td>9.9*</td>
<td></td>
<td>37.8</td>
</tr>
<tr>
<td>Present</td>
<td>15.8</td>
<td>10.9*</td>
<td></td>
<td>97.1</td>
</tr>
<tr>
<td>None</td>
<td>.26</td>
<td>11.5*</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>2.74</td>
<td>11.3*</td>
<td>1.22</td>
<td></td>
</tr>
</tbody>
</table>

* Recalculated from the data given.

(19). The present experiments demonstrate that this is also true of the mammalian collecting tubule. Since there is evidence that the permeability changes caused by the hormone are at the opposite (luminal) border of the cells (7), it is of interest to consider the intermediate steps within the cells which are necessary for lumen action. It has been suggested on the basis of studies of toad bladder that one of these steps is increased production of cyclic 3',5'-AMP (16). Thus it has been shown that cyclic AMP itself mimics the action of vasopressin and that its concentration in tissue is increased following incubation with antidiuretic hormone (6). In the present

studies it has been shown that cyclic 3',5'-AMP has the same action on the permeability to water of the mammalian collecting tubule as does vasopressin. This finding, coupled with the fact that cyclic 3',5'-AMP production in mammalian kidney is increased by vasopressin (1), suggests that this nucleotide is an intermediate in the action of vasopressin in the mammalian renal tubule as well as in the toad bladder.

We wish to thank Dr. Jack Orloff for his many helpful suggestions, and Dr. Clifford Patlak, who derived the equations used to calculate permeabilities.

REFERENCES