Effect of Vasopressin on Electrical Potential Difference and Chloride Transport in Mouse Medullary Thick Ascending Limb of Henle's Loop

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ABSTRACT Medullary thick ascending limbs of Henle's loop of the Swiss-Webster mouse were perfused in vitro with an isotonic perfusate and a Ringer's bathing medium. In five studies, addition of a supramaximal concentration of synthetic arginine vasopressin (AVP) to the bathing medium resulted in an increase in electrical potential difference (PD) from 5.0±1.5 mV, lumen positive, to 10.7±1.4 mV ($P < 0.001$). When AVP was removed, the PD returned to 2.6±0.9 mV ($P < 0.001$), then increased again to 6.9±1.7 mV ($P < 0.01$) when AVP was added a second time. A significant, but submaximal, increase in PD of 2.3±0.6 mV ($P < 0.05$) was observed in five medullary thick ascending limbs when AVP was added to the bathing medium at a concentration of 10 μU/ml. This increase was approximately one-third of the response observed at a concentration of 100 μU/ml in the same tubule. No further increment in PD was observed in five medullary thick ascending limbs when the AVP concentration was increased from 100 to 1,000 μU/ml. In seven thick ascending limbs, the effect of AVP on PD was reproduced by the addition of 8-[*p*-chlorophenylthio]-cyclic 3',5'-adenosine monophosphate to the bathing medium at a final concentration of 0.1 mM. AVP increased unidirectional chloride flux from lumen to bath from 29.3±3.2 to 69.8±6.2 peq/cm per s ($P < 0.01$) in spite of an increase in the lumen positive PD from 1.6±0.5 mV to 7.0±0.6 mV ($P < 0.001$). Unidirectional chloride flux from bath to lumen was not affected by AVP. In another series of experiments, net chloride flux increased from 15.6±3.0 to 41.7±5.3 peq/cm per s ($P < 0.05$) after addition of AVP. The effect of AVP on hydraulic water permeability (Lp) was examined by adding raffinose to the bathing medium in both the presence and the absence of AVP. The calculated Lp of 16±2 nm/s per atm in the absence of AVP, although very low, was significantly different from zero ($P < 0.01$). However, the Lp did not increase significantly when AVP was added to the bathing medium. These results suggest that AVP has a second site of action in the kidney to increase chloride transport by the medullary thick ascending limb in addition to its well-known effect on the water permeability of the collecting tubule. The former effect would contribute to urinary concentrating ability by increasing the axial osmotic gradient in the renal medulla.

INTRODUCTION

It is well established that vasopressin administration results in the production of a concentrated urine by increasing the water permeability of the collecting tubule system, thereby allowing osmotic equilibration between the luminal fluid and the hypertonic papillary interstitium (1). However, several investigators have suggested that vasopressin might have a second site of action that results in the accumulation of medullary solute and thereby increases the osmotic gradient for fluid absorption (2, 3). Support for this thesis has come from studies demonstrating a vasopressin-sensitive adenyl cyclase in the thick ascending limb of Henle's loop of the rabbit (4), the rat (5), and the mouse (6), although the functional significance of the arginine vasopressin (AVP)-induced[1] increase in adenyl cyclase activity could not be assessed. The present study was undertaken to examine the possible effect of vasopressin on solute or water transport by the medullary thick ascending limb of the Swiss-Webster mouse.

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[1] *Abbreviations used in this paper*: AVP, arginine vasopressin; ClPheS-cAMP, 8-[*p*-chlorophenylthio]-cyclic AMP; Lp, hydraulic water permeability; PD, electrical potential difference; V_0, perfusion rate.

METHODS

Male Swiss-Webster mice, aged 2 mo, were killed either by the intraperitoneal injection of pentobarbital (Abbott Laboratories, North Chicago, Ill.) or by a blow to the head, then bilateral nephrectomies were performed. The kidneys were sliced into quarters, placed in a chilled Ringer's solution, and thin sheets of tissue were peeled off the cut surface of the kidney. Thick ascending limbs were then isolated by free-hand dissection from the thin sheets of tissue. Medullary thick ascending limbs were distinguished from cortical thick ascending limbs by cutting away the cortex before final dissection. Dissection time was 30 min or less in all cases.

Standard techniques for perfusion, as first described by Burg et al. (7) were followed. Briefly, the isolated tubules were transferred to an acrylic chamber containing a Ringer's bathing medium mounted on the stage of an inverted microscope (Unitron Instruments, Inc., Woodbury, N. Y.). Once in the perfusion chamber, both ends of the tubule were attached to glass pipettes, the ends insulated with Sylgard 184 (Dow Corning Corp., Midland, Mich.), and the lumen cannulated. The tubule length averaged 0.4 mm between the Sylgard seals. Unless otherwise indicated, the dissecting and bathing medium used in all studies contained 115 mM NaCl, 25 mM $NaHCO_3$, 10 mM Na acetate, 5.0 mM KCl, 1.2 mM NaH_2PO_4, 1.0 mM $CaCl_2$, 1.2 mM $MgSO_4$, and 5.5 mM glucose (total 290 mosmol/kg H_2O) to which was added 5% calf serum vol/vol. The pH of the bathing medium was maintained at 7.40±0.05 by gently blowing 20% CO_2–80% O_2 over the surface of the chamber. The adequacy of pH control was examined periodically during each experiment with a blood gas analyzer (model 113, Instrumentation Laboratory, Inc., Lexington, Mass.). The bathing medium was exchanged every 10 min to prevent significant changes in osmolality or pH. The temperature of the chamber was maintained at 37±0.5°C with a thermoregulator (Yellow Springs Instrument Co., Yellow Springs, Ohio) attached to a heating coil embedded in the chamber. The rate of perfusion was controlled in all cases by attaching a microliter syringe driven by a syringe pump (model 355, Sage Instruments Div., Orion Research, Inc., Cambridge, Mass.) to the back of the perfusion pipette.

Electrical potential difference. For this series of experiments, the medullary thick ascending limbs were perfused with an isotonic solution containing 125 mM NaCl, 25 mM $NaHCO_3$, 2.5 mM K_2HPO_4, 1.0 mM $CaCl_2$, and 1.2 mM $MgSO_4$. The pH of the perfusate was adjusted to 7.4 by gassing with CO_2 before use. The electrical potential difference (PD) was measured across the epithelium by placing one bridge containing 0.16 m NaCl in 4% agar in the bathing medium and attaching another to the back of the perfusion pipette. The bridges were connected via calomel reference cells to a digital electrometer (model 615, Keithley Instruments, Inc., Cleveland, Ohio), and the PD registered on a chart recorder (model G-2000, Varian Associates, Inc., Palo Alto, Calif.) attached to the output of the electrometer.

Isolated tubules were perfused for 2 h to allow them to recover from the possible effects of endogenous vasopressin. The bathing medium was then exchanged for one identical in composition except for the addition of AVP (Sigma Chemical Co., St. Louis, Mo.) or 8-[*p*-chlorophenylthio]-cyclic 3′,5′-adenosine monophosphate (ClPheS-cAMP) (ICN Nutritional Biochemicals, Cleveland, Ohio). Unless stated otherwise, tubules were exposed to these agents for 20 min and then the bathing medium was replaced with the usual Ringer's medium to allow recovery. In most cases, the ability to reduce the PD to zero was tested by cooling the chamber to 15°C at the conclusion of the experiment.

The PD was examined in 18 thick ascending limbs. In five studies after AVP was added to the bathing medium and removed to allow recovery, it was then added a second time. In six studies, AVP was added sequentially at different concentrations then removed to allow recovery. In seven studies, AVP and ClPheS-cAMP were added separately to the same tubule with an intervening recovery period. The reported values for the PD during control and experimental periods represent the average of the observed PD over the last five 1-min intervals of that period.

For most experiments, [^{14}C]inulin was added to the perfusate as a volume marker to determine perfusion rate and volume absorption. The rate of perfusion was calculated from the rate of appearance of the isotope in the collected fluid and its measured concentration in the perfusate. The rate of collection was determined from the time interval and the collected volume as measured with a constant-bore glass capillary tube. Volume absorption (J_v) was calculated as the difference between the rates of perfusion and collection.

The composition of the perfusate was altered in several experiments using a glass pipette mounted inside the perfusion pipette. In five studies, the usual perfusate was alternated with one containing 10 μM furosemide. The PD fell within 1 min or less after the addition of furosemide, then remained stable for up to 10 min. Recovery, although rapid, frequently required several minutes to achieve a stable PD. In another six experiments, the bulk of the chloride ion or sodium ion was removed simultaneously from the bathing medium and perfusate and the resultant PD observed. These bathing and perfusing solutions contained 2.5 mM K_2HPO_4, 1.0 mM $CaCl_2$, 1.2 mM $MgSO_4$, and, in addition, either 0.15 M NaCl, 0.15 M choline chloride, or 0.118 M Na_2SO_4, or, in five cases, 0.15 M $NaNO_3$. The bathing medium also contained 5% calf serum. Except for the solution containing choline chloride, the PD achieved a stable level within 2 min and remained stable for 10 min after the change in the solution. With the choline chloride, as much as 10 min was required before a stable value was obtained. The effect of each solution on PD was readily reversible.

Chloride transport. Unidirectional chloride fluxes and net chloride transport were measured in tubules isolated and perfused as previously described. In these experiments, however, the perfusate was identical to the Ringer's bathing medium except that calf serum was not added. The rate of chloride flux from lumen to bath was examined in five studies by adding ^{36}Cl (20 μCi/ml) to the perfusate. Because volume absorption was not significantly different from zero, flux was calculated from the formula (8):

$$J_{Cl,lb} = \frac{V_0 C_0}{L} \ln \frac{C_0^*}{C_L^*} ,$$

where $J_{Cl,lb}$ is the unidirectional flux of chloride from lumen to bath in picoequivalents per centimeter per second, V_0 is the perfusion rate in cubic centimeters per second, C_0 is the concentration of chloride in the perfusate in picoequivalents per cubic centimeter, L is the tubule length in centimeters, C_0^* is the concentration in counts per minute per nanoliter of ^{36}Cl in the perfusate and C_L^* is the concentration in counts per minute per nanoliter of ^{36}Cl in the collected fluid. The rate of perfusion was sufficiently rapid to keep the loss of ^{36}Cl from the lumen in the absence of AVP at 10% or less of the quantity perfused.

Unidirectional flux from bath to lumen was examined in seven experiments by adding ^{36}Cl (20 μCi/ml) to the bathing medium 45 min before the addition of AVP, then maintained at a constant concentration by addition of distilled water (20 μl/5 min) until 45 min after the addition of AVP. In four

of these experiments, the tubule was allowed to recover from the effect of AVP in a Ringer's medium without added AVP for 60 min then ^{36}Cl was reintroduced and the unidirectional flux again measured. Chloride flux from bath to lumen was calculated from the formula (8):

$$J_{Cl,bl} = \frac{V_0 C_b}{L}\left[\frac{C_L^*}{C_b^*}\right],$$

where $J_{Cl,bl}$ is the unidirectional flux from bath to lumen in picoequivalents per centimeter per second, C_b is the concentration of chloride in the bath in picoequivalents per cubic centimeter, C_b^* is the concentration in counts per minute per nanoliter of ^{36}Cl in the bath, and the other symbols are as previously defined. C_L^* was <5% of C_b^* in all the reported experiments.

Unidirectional flux from bath to lumen was used to calculate the apparent permeability coefficient, assuming that the flux in this direction is passive and that the permeability is not a function of PD, and that the constant field assumption applies (9):

$$P_{Cl} = -J_{Cl,bl}RT(1 - e^{EZF/RT})/C_bEZF,$$

where P_{Cl} is the apparent permeability coefficient, R is the gas constant, T is the absolute temperature, E is the voltage across the epithelium, Z is the valence, and F is Faraday's constant. The voltage used for this calculation was the average PD observed during the entire period of collection.

Net chloride transport was also assessed by adding ^{36}Cl to a Ringer's solution of the composition given above and using this solution for both the bathing medium and perfusate. Because the specific activity of ^{36}Cl was identical in both the perfusate and the bathing medium, changes in the concentration of ^{36}Cl in the collected fluid reflect changes in the chemical concentration of chloride. Since volume absorption was again not significantly different from zero, net chloride absorption was calculated as

$$J_{Cl}^* = V_0C_0(1 - C_L^*/C_0^*)/L,$$

where J_{Cl}^* is the apparent net chloride absorption in picoequivalents per centimeter per second and the other symbols are as previously defined.

Hydraulic water permeability. The effect of AVP on hydraulic water permeability (Lp) was examined in five medullary thick ascending limbs (0.45–0.70 mm in length) by perfusing the tubule with the same solution used in the PD experiments and generating an osmotic gradient by adding 160 mM raffinose to the usual bathing medium. Tubules were initially placed in a Ringer's medium of the composition described above, then the bathing medium was replaced with one containing Ringer's solution plus 160 mM raffinose for 40 min, and then the bathing medium was replaced by one containing 160 mM raffinose in Ringer's solution plus 100 μU/ml AVP. The tubule was exposed to the latter solution for 60 min, then the bathing medium was replaced by Ringer's solution alone. Volume absorption during each period was determined as described above.

Aliquots of the fluid collected during exposure to raffinose and raffinose plus AVP were removed for measurement of osmolality using the technique of Ramsay and Brown (10). Lp was calculated from the formula:

$$Lp = \frac{J_v}{\Delta\pi},$$

where Lp is the hydraulic water permeability in nanometers per second per atmosphere, J_v is the volume absorption in cubic centimeters per second per square centimeter surface area, and $\Delta\pi$ is the osmotic pressure gradient across the epithelial layer in atmospheres (atm). The surface area was calculated from the length of the tubule as measured with an eyepiece micrometer in the microscope and an assumed internal Diam of 15 μm (the average internal diameter of 10 mouse medullary thick ascending limbs). The osmotic pressure gradient, $\Delta\pi$, was calculated as the difference between the osmolality of the bathing medium and the arithmetic mean of the measured osmolalities of perfused and collected fluids. A more rigorous formula for the calculation of mean osmotic gradient and Lp has been applied to the collecting tubule (11, 12). However, this formula requires that the total luminal solute must remain unchanged and that the reflection coefficients for solutes contributing to the gradient must be 1.0. The former requirement does not apply to the thick ascending limb and the latter requirement has not been examined in this segment. Therefore, in the absence of specific knowledge to the contrary, the change in intraluminal osmolality has been assumed to be linear with tubule length for the present calculation. Because dilution of the luminal contents only accounts for 20% or less of the total osmotic gradient (see Results), this method for calculating the mean luminal osmolality will not introduce a major error.

The osmolality of bulk solutions was measured with a vapor pressure osmometer (Wescor Inc., Logan, Utah).

Student's *t* test for paired and unpaired data was used to test for statistical significance (13).

RESULTS

Medullary thick ascending limbs of the mouse are similar in appearance to those obtained from the rabbit (14), and distinctly different from the mouse medullary collecting tubule, in which considerable variation in cell size is noted (Fig. 1).

PD. As in the rabbit thick ascending limb, the PD was found to be lumen positive and in nine tubules, it reached +25±1 mV under stop-flow conditions, a value similar to that previously reported for the rabbit thick ascending limb (8, 14). The effects of ion substitutions in the bathing medium and perfusate on the PD (Table I) are similar to those previously observed in the rabbit (8, 14) and indicate that the PD is largely dependent on the presence of chloride ion. The PD was also inhibitable by the addition of 10 μM furosemide to the perfusate in five experiments, reducing the PD from +6.7±1.0 to +1.9±0.3 mV ($P < 0.01$) after addition of furosemide, and with a recovery to +7.0±1.3 mV ($P < 0.02$) after furosemide was removed, results that are again similar to those found in the rabbit (15, 16).

When AVP was added to the bathing medium at a constant rate of perfusion, the PD remained relatively stable for 1–3 min, then increased over 10–15 min to reach a stable plateau as illustrated in Fig. 2. When AVP was removed from the bathing medium, the PD gradually returned to the base line over 60–120 min. In some cases, the PD began to fall immediately

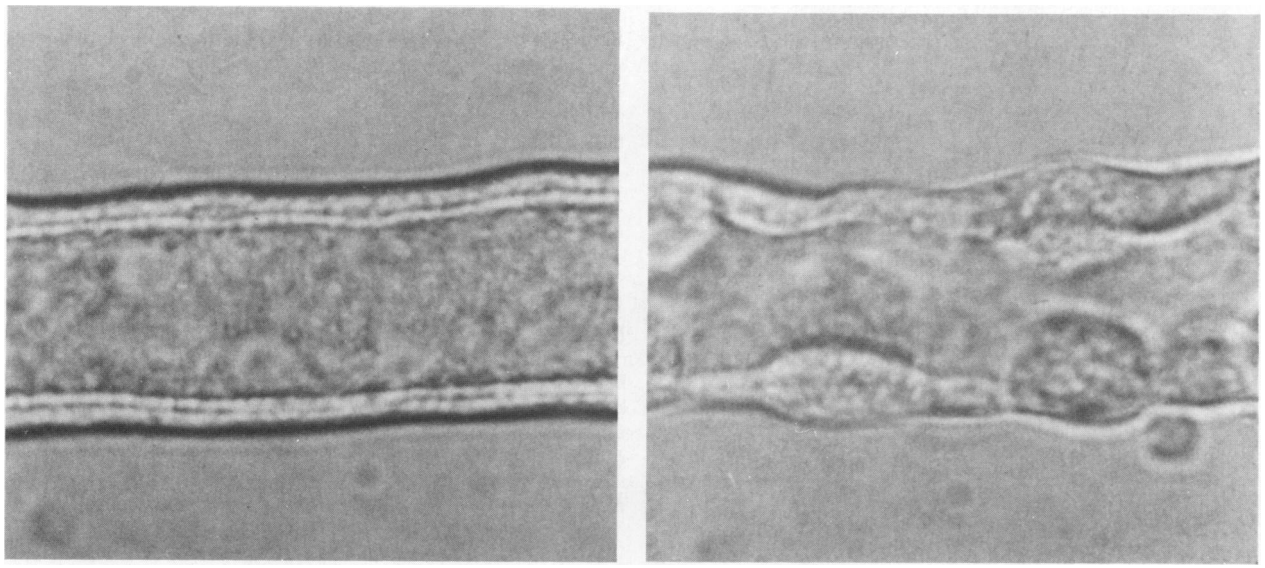

FIGURE 1 Morphology of perfused mouse (left) medullary thick ascending limb and (right) medullary collecting tubule. The uniform cell type of the thick ascending limb is apparent as is the variation in cell size observed in the collecting tubule. The average diameters of the two segments were 31 μm o.d. and 21 μm i.d. for the collecting tubule, and 26 μm o.d. and 15 μm i.d. for the thick ascending limb. ×600.

after AVP was removed, whereas in others the PD remained at the plateau level for several minutes before beginning to decline.

In five experiments, AVP was added to the bathing medium at two separate times (Fig. 3) and each addition resulted in an increase in PD (Table II). The increase in PD of 5.7±0.3 mV observed with the first exposure to AVP is highly significant ($P < 0.001$) as is the decrease of 8.1±0.8 mV ($P < 0.001$) during the recovery period. The second addition of AVP also resulted in a significant increase in PD of 4.3±0.9 mV ($P < 0.01$) but the response was less with the second exposure to AVP in each case.

The effect of lower concentrations of AVP was tested in six medullary thick ascending limbs by adding different concentrations of AVP to the bathing medium. Because of the prolonged recovery time noted above, AVP was added sequentially in increasing concentration and no recovery period was obtained until the end of the experiment. Since the response to an AVP concentration of 10 μU/ml developed slowly, the tubules were exposed to this concentration of AVP for 40 min rather than the usual 20 min. Furthermore, the exposure time for an AVP concentration of 100 μU/ml was increased to 30 min. Nevertheless, in some experiments (Fig. 4), no clear plateau was seen before the next concentration of AVP was added. Thus the re-

TABLE I
Effect of Ion Substitutions on PD

Major solute*		PD
		mV
0.15 M NaCl	(6)	+7.1±1.1
0.15 M choline Cl	(6)	+10.5±1.7‡
0.15 M $NaNO_3$	(5)	+0.6±0.2‡
0.118 M Na_2SO_4	(6)	+0.4±0.2‡

* Each solution contained other solutes as well, as given in Methods. The substitutions were made simultaneously in both bathing medium and perfusate. The number in parentheses is the number of experiments.
‡ $P < 0.01$ as compared with 0.15 M NaCl in the same tubule.

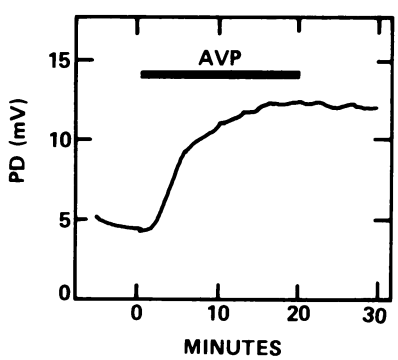

FIGURE 2 Effect of AVP on the PD of a mouse medullary thick ascending limb. The horizontal bar represents the duration of exposure to AVP. The addition of AVP resulted in an initial slight decrease in PD, then, after a slight delay, a substantial increase that was sustained after AVP was removed.

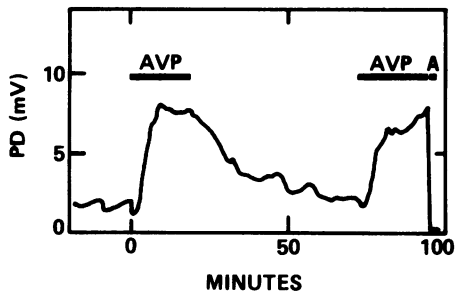

FIGURE 3 Effect of AVP on a mouse medullary thick ascending limb. AVP was added to the bathing medium at two separate times, as indicated by the horizontal bars. The AVP concentration was 250 μU/ml. At point A, the temperature was reduced to 15°C, then the tubule was removed from the pipettes.

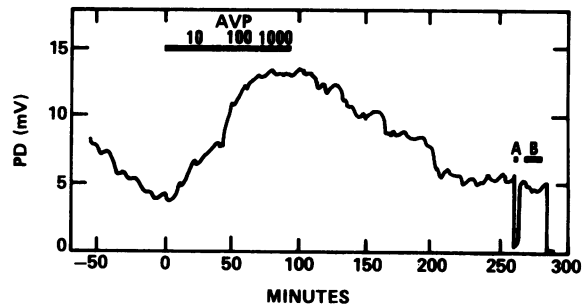

FIGURE 4 Effect of different concentrations of AVP on the PD of a mouse medullary thick ascending limb. The horizontal bars indicate the duration of exposure to AVP, and the numbers above the bar are the concentrations of AVP in microunits per milliliter. During period A, the temperature was reduced to 15°C. During period B, the bathing medium contained 0.1 mM ouabain. After the ouabain was replaced with Ringer's medium, the tubule was removed from the pipettes.

ported change in PD may be an underestimate of the maximal effect attainable. The PD observed over the last 5 min of exposure to each concentration are reported in Table III. The response to a concentration of 10 μU/ml ranged from substantial (experiment 5) to trivial (experiment 3), but it was statistically significant for the five experiments ($P < 0.05$). The response to a concentration of 10 μU/ml was 35% of the response observed at 100 μU/ml in the same tubule, but, as indicated above, this may represent an underestimate of the true value. An AVP concentration of 100 μU/ml is apparently maximal because there was no significant increase in PD when the concentration was increased from 100 to 1,000 μU/ml in five studies (Table III).

The ability to reproduce these changes in PD with ClPheS-cAMP at a concentration of 0.1 mM was tested in seven thick ascending limbs (Fig. 5). The PD increased from a base line of 5.5±0.6 mV to 14.9±1.4 mV ($P < 0.001$) after the addition of ClPheS-cAMP then recovered to 4.6±0.9 mV ($P < 0.001$) after ClPheS-cAMP was removed. The increment in voltage after the addition of ClPheS-cAMP, 9.4±1.3 mV, was somewhat greater than the increment of 6.7±1.0 mV observed after the addition of AVP (100 μU/ml) in the same tubule.

When the data of all studies reported thus far are combined, 18 medullary thick ascending limbs were exposed to AVP at a concentration of 100 μU/ml or greater. Addition of AVP to the bathing medium resulted in an increase in PD from 4.7±0.6 mV to 10.9±0.7 mV ($P < 0.001$) and the PD recovered to 3.9 ±0.5 mV ($P < 0.001$) after AVP was removed.

Because the rate of perfusion can affect the PD in the rabbit (8, 14), the possible role of this variable was excluded in 10 of the 18 experiments. Although there was some variation in perfusion rate during the course of individual experiments (±20% or less), there

TABLE II
Effect of Two Separate Exposures to AVP on the PD of Mouse Medullary Thick Ascending Limb

Experiment	AVP concentration	PD			
		Base line	AVP	Recovery	AVP
	μU/ml	mV			
1	1,000	7.0	12.5	3.5	8.5
2	1,000	9.7	15.1	5.5	11.7
3	250	2.1	7.7	2.2	7.5
4	250	4.9	9.9	0.8	1.8
5	250	1.5	8.3	1.0	5.0
Mean		5.0	10.7	2.6	6.9
SEM		±1.5	±1.4	±0.9	±1.7
P*			<0.001	<0.001	<0.01

* Statistical evaluation for a paired comparison between adjacent columns.

TABLE III
*Effect of Different Concentrations of AVP on PD**

Experiment	Base line	AVP 10 μU/ml	AVP 100 μU/ml	AVP 1,000 μU/ml	Recovery	10 μU/ml minus base line	1,000 minus 100 μU/ml
				mV			
1	2.7	—	12.7	14.1	8.3	—	1.4
2	10.6	13.7	16.8	—	7.7	3.1	—
3	1.7	2.0	6.2	6.6	1.7	0.3	0.4
4	1.8	3.5	8.6	8.4	1.6	1.7	−0.2
5	4.2	8.1	13.2	13.1	5.5	3.9	−0.1
6	6.1	8.5	10.7	9.2	4.7	2.4	−1.5
Mean	4.5	7.2	11.4	10.3	4.9	2.3	0.0
SEM	±1.4	±2.1	±1.5	±1.4	±1.2	±0.6	±0.5
P‡						<0.05	NS

* AVP was added sequentially from low dose to high dose in all cases.
‡ Statistical evaluation for a paired comparison between the indicated columns.

was no significant change in the perfusion rate for the group as a whole (Table IV). Since PD for the group increased from 3.9 to 10.3 mV while the perfusion rate was constant, changes in flow rate cannot provide an explanation for the present results. Volume absorption was not significantly different from zero either in the presence or in the absence of AVP.

The effect of intraluminal AVP was examined in four experiments. Addition of 200 μU/ml AVP to the perfusate resulted in an insignificant change in PD from 3.7 ±0.4 mV during the base-line period to 3.6±0.5 mV after 20 min of intraluminal AVP exposure. When 100 μU/ml AVP was added to the bathing medium of the same tubules, the PD began to increase within 2–3 min and reached a value of 8.0±0.7 mV within 15 min, a result similar to that previously observed in other experiments.

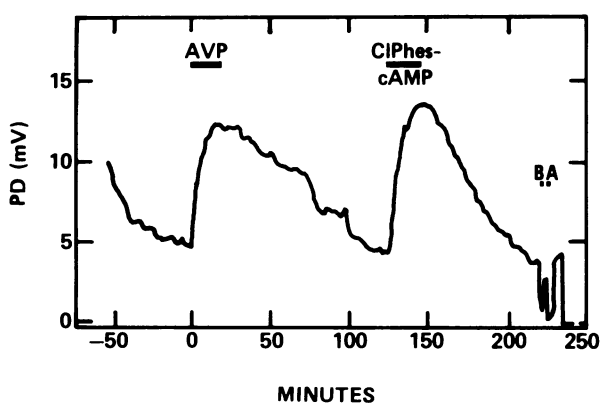

FIGURE 5 Effect of AVP (100 μU/ml) and ClPheS-cAMP (0.1 mM) on the PD of a mouse medullary thick ascending limb. The duration of exposure to each drug is indicated by the horizontal bar. During period A, the temperature was reduced to 15°C, and during period B, 1 mM ouabain was added to the bathing medium.

The PD fell to zero when the bathing medium was cooled to 15°C whether the PD was at its peak during exposure to AVP (Fig. 3) or at its base line (Figs. 4 and 5). Furthermore, the PD continued to be largely dependent of the presence of chloride ions during exposure to AVP. In five experiments in which the PD had increased from 2.8±0.6 to 7.6±1.0 mV ($P < 0.001$) in response to AVP, replacement of the bathing medium and perfusate with the previously described $NaNO_3$ solution resulted in a fall in PD to 0.6±0.2 mV, a value that is virtually identical to that observed in the absence of AVP (Table I). The PD rapidly recovered in these experiments to 7.3±0.7 mV when the $NaNO_3$ solution was replaced with the usual solutions containing chloride.

Chloride transport. The effects of AVP (100 μU/ml) on the unidirectional chloride fluxes from lumen to bath and from bath to lumen were examined in separate groups of tubules and the results are illustrated in Fig. 6. The addition of AVP markedly increased the rate of chloride flux from lumen to bath in five tubules in spite of an increase in the lumen positive PD from 1.6±0.5 to 7.0±0.6 mV ($P < 0.001$) (Table V). Chloride fluxes from bath to lumen and the chloride permeability calculated from this flux tended to increase gradually during the experiment and the final values for both are significantly greater than those observed during the base-line and the AVP periods. However, although there was a modest increase in the mean values for chloride permeability and $J_{Cl,bl}$ during exposure to AVP, these differences were not significant when compared with the base-line period (Table VI).

TABLE IV

*PD, Perfusion Rate, and Volume Absorption in 10 Medullary Thick Ascending Limbs**

	Base line		AVP*		Recovery
PD, *mV*					
Mean±SE	3.9±0.6		10.3±0.8		3.5±0.6
P‡		<0.001		<0.001	
Perfusion rate, *nl/min*					
Mean±SE	2.0±0.2		2.0±0.3		1.9±0.3
P‡		NS		NS	
Volume absorption, *nl/mm per min*					
Mean±SE	−0.01±0.04		0.01±0.04		0.01±0.05
P‡		NS		NS	

* The concentration of AVP was 100 μU/ml in all cases except one in which 250 μU/ml was used.

‡ Statistical evaluation is for a paired comparison of adjacent columns.

Net chloride flux was examined in another four experiments by adding ^{36}Cl to both sides of the epithelium and using the concentration of ^{36}Cl as a measure of the chemical concentration of chloride. These data are reported in Table VII. At a constant rate of perfusion, the addition of AVP increased the PD, reduced the concentration of chloride in the collected fluid, and increased net chloride flux as compared with the base-line and recovery periods.

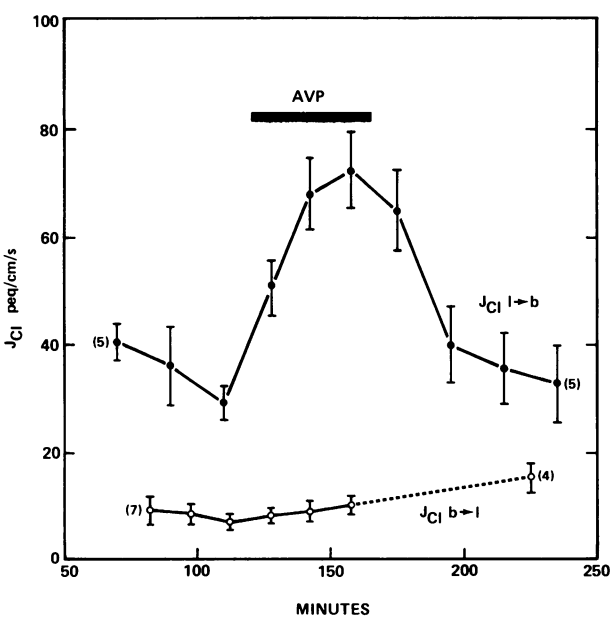

FIGURE 6 Effect of AVP (100 μU/ml) on the unidirectional chloride flux. The number of tubules is indicated in parentheses. The horizontal bar indicates the duration of exposure to AVP. The mean±SE of the observed flux for a collection period is plotted against the time in minutes after the death of the animal.

Hydraulic water permeability. Lp was estimated in six medullary thick ascending limbs by adding 160 mM raffinose to the bathing medium to generate an osmotic gradient. Volume absorption in these tubules in the absence of the imposed gradient was 0.01±0.04 nl/mm per min and increased by 0.21±0.02 nl/mm per min ($P < 0.01$) when raffinose was added to the bathing medium (Table VIII). When AVP was added to the bathing medium along with the raffinose, there was no further increase in the rate of volume absorption as compared with that for raffinose alone.

The osmolality of the collected fluid was significantly lower than the perfusate osmolality (285±2) both with raffinose alone ($P < 0.01$) or with AVP plus raffinose ($P < 0.01$). The osmolality of the collected fluid during exposure to AVP was lower than with raffinose alone (Table VIII). The total osmolar absorption, calculated as $(V_0C_0 - V_LC_L)/L$ where V_0 and V_L are the flow rates, and C_0 and C_L are the osmotic concentrations, of perfusate and collected fluids respectively, and L is the tubule length, demonstrated a significant increase in solute absorption from 37.3±7.1 to 50.7±10.3 posmol/cm per s after addition of AVP. Lp was calculated from the volume absorption and mean osmotic gradient (see Methods), but since the gradient reflects both raffinose and other solutes, and the reflection coefficients were not determined, it only represents an estimate. The calculated Lp of 16±2 nm/s per atm observed with raffinose alone is significantly different from zero ($P < 0.01$) and is somewhat greater than the 0–8-nm/s per atm previously reported in the rabbit thick ascending limb (8, 14). However, the Lp was not affected by the addition of AVP.

TABLE V
Effect of AVP on Chloride Flux from Lumen to Bath

	Base line	AVP	Recovery
V_0, *nl/min* (5)*	7.8±0.8	7.0±1.1	6.8±0.8
PD, *mV* (5)	+1.6±0.5	+7.0±0.6‡	+1.9±0.8
$J_{Cl,lb}$, *peq/cm per s* (5)	29.3±3.2§	69.8±6.2	32.9±7.3‖

* The number of experiments is given in parentheses. The tubule length was 0.46±0.06 mm.
‡ $P < 0.001$ when compared with the base-line and recovery periods.
§ $P < 0.01$ when compared with the AVP period.
‖ $P < 0.02$ when compared with the AVP period.

DISCUSSION

Numerous investigators have observed that the increase in medullary solute concentration seen in antidiuresis as compared with water diuresis, is a consequence of an increased solute content as well as a reduced water content (2, 3, 17–21). By using graded doses of lysine-vasopressin, Atherton et al. (3) further observed that the reduction in free water clearance occurred at a lower dose of the hormone than that required to increase medullary solute content. This finding led them to suggest that vasopressin has two sites of action in the kidney, with one site primarily affecting water permeability and the other site affecting medullary solute content.

The reduced medullary water content seen in antidiuresis apparently results from a shift in the location of water absorption from the terminal medullary collecting tubule to more proximal sites, especially the cortical collecting tubule, in the presence of vasopressin (22). The increase in medullary solute content has not been so readily explained, however, and investigators have not been able to distinguish between reduced solute removal, presumably mediated by changes in medullary blood flow, and increased net solute addition. Furthermore, the increased solute addition, if it occurs, might result from increased active transport by some medullary segment of the nephron or from increased urea recycling and passive sodium absorption (23). Recent observations demonstrating a vasopressin-sensitive adenyl cyclase in the thick ascending limb of Henle's loop in several species (4–6) have provided some support for the notion that vasopressin might increase medullary solute content, in part, by stimulating active transport by the thick ascending limb. However, the functional significance of the vasopressin-induced increase in adenyl cyclase activity could not be assessed in these studies.

In the present experiments, the functional effect of AVP was tested in the mouse medullary thick ascending limb. This segment in the mouse was found to resemble the previously examined rabbit thick ascending limb with respect to its morphologic appearance, the direction of the PD, the effect of ion substitution and furosemide on the PD, and, at least during the base-line and AVP periods, the calculated chloride permeability (8, 14). The rate of net chloride flux, 15.6 peq/cm per s (Table VII) is also quite close to the value of 13.3 peq/cm per s observed in the rabbit by Stokes (24) under very similar experimental conditions. The Lp observed in the mouse is slightly higher than that seen in the rabbit (8, 14), but the lack of effect of AVP on the Lp of this segment is consistent with the observation that AVP does not affect diffusional water permeability in the rabbit (25).

The present results demonstrating an effect of AVP

TABLE VI
Effect of AVP on Chloride Flux from Bath to Lumen

	Base line	AVP	Recovery
V_0, *nl/min*	7.9±0.9 (7)*	8.2±1.3 (7)	6.7±1.2 (4)
PD, *mV*	+5.2±0.5 (7)	+8.3±0.6 (7)‡	+2.1±0.1 (4)
$J_{Cl,bl}$, *peq/cm per s*	6.9±1.3 (7)	9.5±1.9 (7)	15.6±2.7 (4)§
P_{Cl}, *cm/s* × 10^5	1.1±0.2 (7)	1.4±0.3 (7)	2.6±0.5 (4)§

* The number of experiments is given in parentheses. Tubule length for seven experiments was 0.45±0.03 mm.
‡ $P < 0.01$ as compared with base-line and recovery periods.
§ $P < 0.05$ as compared with base-line and AVP periods.

TABLE VII
*Effect of AVP on Net Chloride Transport in Four Mouse Medullary Thick Ascending Limbs**

	Base line	AVP	Recovery
V_0, *nl/min*	5.3±1.0	5.3±1.2	5.4±0.9
PD, *mV*	+4.3±0.6‡	+10.5±1.8	+2.7±0.6§
$[Cl]\frac{\text{collected}}{\text{perfused}}$	0.92±0.02‡	0.79±0.02	0.95±0.01‖
$[Cl]\frac{\text{collected}}{\text{bath}}$	0.92±0.01‡	0.81±0.03	0.98±0.02‖
J_{Cl}*, *peq/cm per s*	15.6±3.0‡	41.7±5.3	10.2±2.9‖

* The tubule length was 0.51±0.02 mm.
‡ $P < 0.05$ when compared with the AVP period.
§ $P < 0.02$ when compared with the AVP period.
‖ $P < 0.01$ when compared with the AVP period.

on the PD, on the unidirectional chloride flux from lumen to bath, and on net chloride transport provide functional evidence that AVP can modulate the rate of transport in the thick ascending limb. Since the PD continued to be largely dependent on the presence of chloride ions and the increased rate of transport occurred against both a chemical (Table VII) and an electrical gradient, active chloride transport was apparently stimulated by AVP. The ability to reproduce the effect on PD by ClPheS-cAMP indicates that the effect of AVP on chloride transport is mediated by cAMP.

There is certainly precedent for an effect of AVP on solute transport, because AVP increases PD and sodium transport in amphibian epithelia (26, 27) and, at least transiently, in isolated rabbit collecting tubules (28, 29). The effect of AVP on solute transport by medullary thick ascending limbs differs from the response seen in these epithelia, however, since chloride is the actively transported species, rather than sodium, and since AVP makes the PD of the apical side more positive rather than more negative. The effect of AVP in the mouse thick ascending limb also differs from the response seen in these epithelia in that whereas solute transport is apparently affected in all cases, water transport is not changed in the medullary thick ascending limb. Although unusual, this finding is not unique since a similar dissociation of the effect of AVP on solute and water transport is observed in the aquatic toad, *Xenopus laevis* (30, 31), and can be produced pharmacologically in the toad bladder (32, 33).

The possible role of AVP in solute transport by the medullary thick ascending limb has previously

TABLE VIII
Effect of AVP on L_p and Osmolar Absorption

Experiment . . .	1	2	3	4	5	6	Mean±SE
Tubule length, *mm*	0.50	0.48	0.45	0.65	0.55	0.70	0.56±0.04
Raffinose							
V_0, *nl/min*	0.8	1.9	1.9	2.5	2.6	6.0	2.6±0.7
Collected osmolality	251	236	232	234	273	252	246±6
J_v, *nl/mm per min*	0.17	0.25	0.15	0.26	0.25	0.17	0.21±0.02
L_p, *nm/s per atm*	13	19	9	20	20	13	16±2
Osmolar absorption, *posmol/cm per s*	15.3	42.3	43.9	38.7	20.2	63.2	37.1±7.1
AVP and raffinose							
V_0, *nl/min*	1.1	1.8	2.0	2.8	1.8	6.1	2.6±0.7
Collected osmolality	232	214	192	212	273	237	227±11*
J_v, *nl/mm per min*	0.32	0.27	0.04	0.40	0.21	0.10	0.22±0.06
L_p, *nm/s per atm*	23	19	3	29	18	7	17±4
Osmolar absorption, *posmol/cm per s*	29.0	53.9	71.1	53.0	14.9	82.0	50.7±10.3‡

* $P < 0.02$ when compared with raffinose period.
‡ $P < 0.05$ when compared with raffinose period.

been examined with the perfused tubule technique. This study found that vasopressin had no effect on the PD of rabbit medullary thick ascending limb (25), a result clearly different from that observed in the present study. The reason for these differing findings is not certain, but several differences exist between the present and the previous studies. In the present study, the tubules were perfused for 2 h to allow any effect of endogenous vasopressin to dissipate, whereas this apparently was not done in the previous study. Furthermore, the bathing medium in the present study was an artificial one containing only a small amount of serum as opposed to serum alone used in the previous study, a difference that could be important if the serum contained vasopressin. Species differences may also be important since Chabardés et al. (6) have found that adenyl cyclase activation by vasopressin in the medullary thick ascending limb is some threefold greater in the mouse than in the rabbit. The difference in adenyl cyclase activation may have functional significance since the AVP-induced adenyl cyclase activation in cortical collecting tubules is sixfold greater in the mouse than in the rabbit (6), and the increase in Lp after exposure to AVP in mouse cortical collecting tubules (34) is also three- to tenfold higher than that observed in the rabbit collecting tubule at the same temperature (11, 35, 36).

Because AVP affects the activity of adenyl cyclase in both the medullary and cortical portions of the thick ascending limb, a reduction in the concentration of sodium chloride might be anticipated in the early distal tubule in vivo during antidiuresis as compared with water diuresis. This comparison has been made by only a few investigators. Johnston et al. (37), in a study using rats with hereditary diabetes insipidus, observed that the osmolality of early distal tubular fluid was increased, rather than decreased, in antidiuresis. However, as the authors noted, the concentration of sodium was not measured and the increased osmolality probably resulted from an increased concentration of urea as a consequence of increased medullary urea recycling during antidiuresis. Schnermann et al. (38), using the same strain of rat, did measure sodium and found that neither the fractional delivery nor the concentration of sodium was changed by vasopressin administration. The application of these data to the present question is complicated, however, by the reduced responsiveness to vasopressin recently observed in thick ascending limbs obtained from this strain of rat (39). Antoniou et al. (40) found that both fractional delivery and concentration of sodium were increased in the early distal tubule after vasopressin administration to dogs. Although they concluded that this might reflect an AVP-induced inhibition of sodium transport, these authors also pointed out that the increased sodium delivery and concentration in the early distal tubule might reflect increased sodium entry into the loop of Henle during antidiuresis. Because the rate of solute delivery was unknown in the last study (40), the data obtained in the early distal tubule could not provide conclusive evidence for or against a change in active transport by the thick ascending limb. Furthermore, although there is no particular reason to doubt its presence, an AVP-sensitive adenyl cyclase has not yet been demonstrated in the dog thick ascending limb. Thus, although these three studies provide no support for increased sodium chloride absorption by the thick ascending limb in vivo, none exclude this possibility.

In summary, the present study has demonstrated that vasopressin increases the PD and the rate of chloride transport in the medullary thick ascending limb of the mouse, an effect probably mediated by adenyl cyclase activation and the generation of cAMP. Water transport, on the other hand, was not affected by AVP. The effect of AVP to increase solute transport in the mouse is in contrast to the recently observed effect of prostaglandins to decrease solute transport in the rabbit medullary thick ascending limb (24). Although an AVP-responsive adenyl cyclase has not been observed in the human thick ascending limb (41), the present results suggest that AVP might, in some species, contribute to urinary concentration in two ways. In addition to its well-known effect of increasing water permeability of the collecting tubule, vasopressin apparently stimulates sodium chloride absorption by the thick ascending limb as well. According to current models for the urinary concentrating mechanism by Stephenson (42) and Kokko and Rector (23), the latter effect will act to enhance the axial osmotic gradient in the renal medulla and thereby contribute to maximal concentrating ability.

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