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Research Article

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Luminal Vasopressin Modulates Transport in the Rabbit Cortical Collecting Duct

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Abstract

We explored the action of luminal AVP in rabbit CCD perfused in vitro at 37°C. Nanomolar concentrations of luminal AVP induced a sustained hyperpolarization of transepithelial voltage (V_t) in contrast to a transient hyperpolarization caused by basolateral AVP. 10 μ M basolateral ouabain abolished the latter but not the former change in V_t . Despite a sustained hyperpolarization (from -20.7 ± 2.9 to -34.1 ± 4.7 mV; $P < 0.01$), 10 nM luminal AVP only slightly altered net Na^+ and K^+ fluxes (7.6% stimulation and no significant change, respectively). Instead, luminal AVP appeared to modulate an acetazolamide-sensitive electrogenic ion transport because 200 μ M basolateral acetazolamide suppressed the luminal AVP-induced hyperpolarization (percentage of V_t from -50.4 ± 10.8 to -5.1 ± 1.4 ; $P < 0.005$). In terms of water transport, 10 nM luminal AVP did not change hydraulic conductivity (L_p , $\times 10^{-7}$ cm/atm per s) (from 3.9 ± 0.8 to 5.0 ± 1.2), but suppressed the increase in L_p induced by 20 pM basolateral AVP (134.9 ± 19.2 vs. 204.3 ± 21.1 in control; $P < 0.05$). These findings demonstrate distinct luminal action of AVP, suggesting amphilateral regulation of epithelial transport by AVP in the CCD. (*J. Clin. Invest.* 1991. 88:952-959.)
Key words: water transport • transepithelial voltage • sodium reabsorption • potassium secretion • proton secretion

Introduction

In the mammalian kidney, the CCD is an important target in the nephron for the antidiuretic action of AVP, the single most important hormone for regulation of urine production. In this nephron segment, it has become evident that, in addition to the adenylyl cyclase-cyclic AMP system, which mediates the classic V_2 receptor-coupled antidiuretic effect of picomolar AVP, multiple signaling pathways are activated when AVP at nanomolar concentration is applied to either isolated tubules (1) or cells (2) in vitro. Unlike the cyclic AMP system, these additional signalings do not induce a hydroosmotic effect (3, 4), but result in self inhibition of the AVP-induced antidiuretic effect in the CCD (1). Also, these signalings may affect transport of ions such as Na^+ , K^+ , and HCO_3^- (5-7). However, the physiological relevance of these heterogeneous actions of nanomolar

AVP has not been confirmed, because the level of circulating AVP is strictly regulated within the picomolar range, and rarely exceeds this even under serious hypovolemia (8).

On the other hand, it is known that AVP is excreted into urine mainly via glomerular filtration and tubular excretion in the distal nephron (9-11). Importantly, when AVP release is stimulated, the final urine contains intact AVP of the nanomolar range, while plasma AVP level is within the picomolar range (9). Thus, in clarifying the comprehensive role of AVP in urine production, it is of interest to explore whether luminal AVP modulates the epithelial functions of the CCD.

Early in vitro microperfusion studies of the CCD by Grantham and Burg (12) revealed that luminal AVP (~ 500 pM) failed to increase osmotic water permeability. In the amphibian urinary bladder epithelium, an analogue of mammalian collecting duct, AVP had also found not to increase water permeability (13) or to alter Na transport (14) when it was applied from the apical side. In a study using primary-cultured canine CCD cells (15), luminal AVP was found to stimulate prostaglandin synthesis. However, it is unknown whether cultured cells retain the intact apico-basolateral polarity of the CCD in situ. In fact, the luminal actions of bradykinin or prostaglandins found in the cultured CCD cells were not observed in isolated perfused CCDs (6, 16-19). Otherwise, to our knowledge, there are no published data describing an effect of luminal AVP. Given the high urinary AVP concentration and the multipotential of nanomolar AVP mentioned above, this study was undertaken to determine whether AVP has a luminal action other than increasing osmotic water permeability.

Methods

Single CCDs were dissected from kidneys of anesthetized (pentobarbital, intravenously injected) male Japanese white rabbits weighing 1.5-2.5 kg, and perfused in a lucite bath chamber on the stage of an inverted microscope at 37°C using the methods described previously (20). To facilitate luminal perfusate exchange during each experiment, a polyethylene tube (PE-10; Clay Adams, Parsippany, NJ) was inserted in the "B" pipette, which was connected to the CCD lumen for perfusion. The perfusate flow rate was adjusted by hydrostatic pressure. Perfusate exchange was done manually by injecting the perfusate into the polyethylene tube, washing out the preexisting medium in the "B" pipette.

The compositions of the bath medium and isotonic perfusate were as follows (in millimolars): NaCl, 105; NaHCO_3 , 25; Na acetate, 10; Na_2HPO_4 , 2.3; NaH_2PO_4 , 1.0; KCl, 5; CaCl_2 , 1.8; MgSO_4 , 1.0; glucose, 8.3; alanine, 5 (osmolality 300 mOsmol). The composition of hypotonic perfusate was identical to that of the isotonic perfusate except for a lower osmolality (150 mOsmol) due to a lower NaCl concentration (30 mM). Tritium-labeled inulin was added to the hypotonic perfusate as a volume marker. Both the isotonic and hypotonic perfusates contained 0.2 mg/ml food, drug, and cosmetic (FD&C) dye for detection of cell damage and perfusate leakage. Before use, all solutions were bubbled to equilibration at 37°C with a 95% O_2 /5% CO_2 gas mixture to achieve a pH of about 7.40, and a PCO_2 of ~ 40 torr. To keep PCO_2 and pH constant, the bath medium was held in a 60 ml plastic syringe connected to the bath chamber by a polyethylene tube (PE-100; Clay Adams) and exchanged continuously during the experiment at a flow

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rate of 25 ml/h by using a syringe pump (model STC-521; Terumo Inc., Tokyo). Perfusates were also stored in plastic syringes until use. The pH values of the bath medium and perfusate were checked again each time just before use using a pH meter (model M-220; Corning Glass Inc., Corning, NY). Experiments were started after the tubules had been perfused for 50–90 min at 37°C to obtain a stable transepithelial voltage (V_t) and to eliminate any residual actions of endogenous AVP (1).

Measurement of transepithelial voltage (V_t) (mV): voltage between two calomel cell electrodes, each connected by a Ringer-agarose bridge to the bath medium and the perfusate in the “B” pipette, was monitored continuously by an electrometer (model 610C; Keithley Instruments, Cleveland, OH or model MEZ-8201; Nihon Kohden, Tokyo) using the standard technique, and recorded on a chart (model R-202 or R-002; Rikadenki, Tokyo) (20).

Measurement of net Na^+ reabsorption (JNa) and K^+ secretion (JK): Na^+ and K^+ concentrations in the perfusate and collected fluid were measured by ultramicroflame photometry using a microanalysis flame photometer (model AFA-707-D; Apel Co., Tokyo). JNa and JK were determined using the following equation:

$$V_o = V_p/t,$$

$$\text{JNa or JK (pEq/mm tubule/min)} = (K_i - K_o) \cdot V_o/L,$$

where V_o is the collection rate, V_p and t are the volume of the constriction pipette and the collection time, respectively, K_i and K_o are the concentrations of Na^+ or K^+ in the perfusate and collected fluid, respectively, and L is the length of the tubule, which was measured directly by eyepiece reticule at the end of each experiment.

Measurement of osmotic water permeability: hydraulic conductivity (L_p) (centimeters per atmosphere per second), a parameter of lumen-to-bath osmotic water flow, was calculated as follows (1):

$$L_p = (1/RTS) \cdot (1/O_b)^2 \cdot \{O_b \cdot (V_i - V_o) + O_i \cdot V_i \cdot \ln \{(O_b - O_i) \cdot V_i / (O_b \cdot V_o - O_i \cdot V_i)\}\},$$

where R is the gas constant, T is the temperature of the bath medium (degree kelvin), S is the luminal surface area of the tubule calculated from the tubule length and an assumed lumen diameter of 20 μm , O_b and O_i are the osmolalities of the bath medium and perfusate, respectively, and V_o and V_i are the collection rate and perfusion rate ($V_i = V_o \cdot C_o/C_i$, where C_o and C_i are radioactivities [counts per minute per nanoliter] of the perfusate, respectively, determined by a liquid scintillation counter [LSC-671; Aloka, Tokyo]).

As is well known (1), the hydroosmotic response of the rabbit CCD to AVP at 37°C is not stable. In our experiments, L_p increased from a basal level to a transient peak in 25–45 min after addition of AVP to the bath medium. Subsequently in some tubules, a small (10–20% in average) decline in L_p due to an unknown mechanism followed, despite the presence of a constant concentration of AVP. Thus, the peak L_p induced by AVP was defined as the mean of the three largest values during exposure to AVP.

Reagents. Acetazolamide, isoproterenol, glucagon, and ouabain were purchased from Sigma Chemical Co., St. Louis, MO. AVP was purchased from Sigma Chemical Co., and Calbiochem Corp., San Diego, CA. [^3H]Inulin was purchased from New England Nuclear, Boston, MA.

Statistics. Data are represented as mean \pm SE. Unless otherwise specified, one-way analysis of variance (ANOVA) was applied to determine statistical significance. The differences at $P < 0.05$ were considered significant.

Results

Using isotonic perfusate, the effects of various doses of luminal AVP on V_t were examined first. In these experiments where V_t

was measured exclusively, a high perfusion rate (~ 50 nl/min) was chosen and the perfused fluid was collected in a large volume (~ 30 μl) pipette to avoid changes in perfusion rate and perfusion pressure that might affect V_t during collection or exchange of perfusate. As shown in Figs. 1 and 2, V_t remained unchanged after an exchange of perfusate to one containing vehicle alone or 10 pM AVP. AVP at 100 pM occasionally caused a small hyperpolarization, as shown in Fig. 1A, but this was not always observed, and the percentage change of V_t caused by 10 or 100 pM AVP was not significantly different from zero. On the other hand, 1 or 10 nM luminal AVP always induced hyperpolarization of V_t . AVP at 100 nM or 1 μM did not further hyperpolarize V_t , as shown in Fig. 2. Thus, a subnanomolar concentration appeared to be the threshold concentration, and 1 and 10 nM were the half-maximal and maximal concentrations required for the V_t change, respectively. The V_t was restored after the removal of luminal AVP (Fig. 1).

To further evaluate the specificity of this effect of luminal AVP, another polypeptide hormone, glucagon (10 nM), was applied to the lumen, but no change in V_t was observed ($n = 3$, data not shown). Also, when a different segment of the collecting duct, the inner stripe of the outer medullary collecting duct (OMCDi), was perfused ($n = 3$), neither 10 nM luminal AVP nor 10 nM luminal glucagon altered the V_t (data not shown).

The hyperpolarization induced by luminal AVP was obviously distinct from that induced by basolateral AVP as shown in Fig. 3. Basolateral AVP caused a transient hyperpolarization, whereas a sustained hyperpolarization was induced by

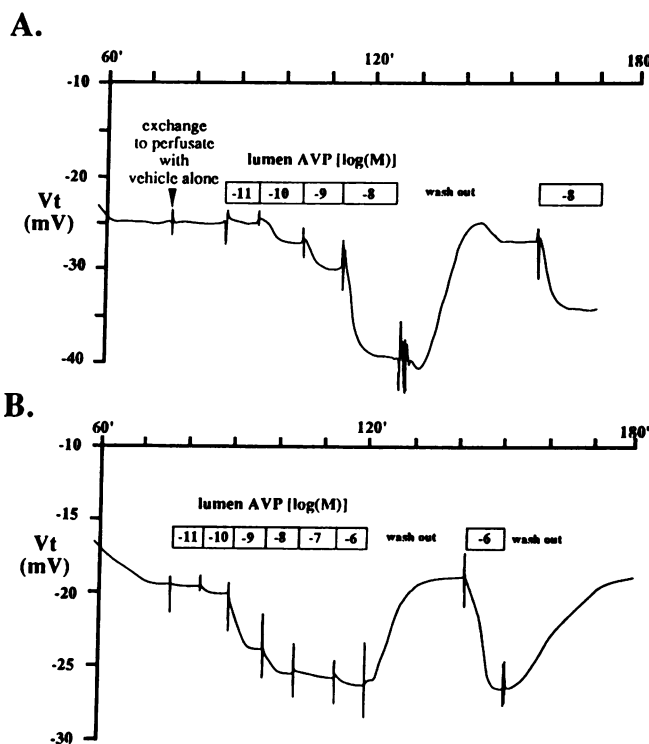


Figure 1. Effect of luminal AVP on V_t in the rabbit CCD perfused in vitro at 37°C. Two original traces of V_t are shown. AVPs from two different sources (Sigma Chemical Co. and Calbiochem Corp.) were used in A and B, respectively. The maneuver to exchange luminal perfusate did not in itself alter V_t . Luminal AVP induced hyperpolarization in a dose-dependent manner in both A and B.

1. **Abbreviations used in this paper:** L_p , hydraulic conductivity; OMCDi, outer medullary collecting duct; V_t , transepithelial voltage.

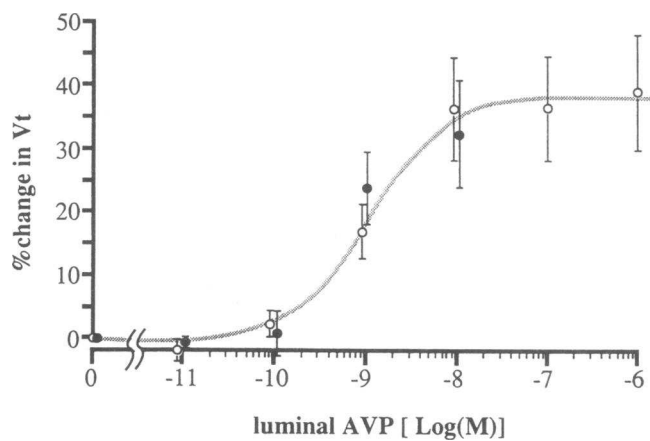


Figure 2. Dose dependency of luminal AVP induced hyperpolarization. Mean percentage V_t were calculated from four to six experiments performed as shown in Fig. 1. Percentage V_t was calculated as: $(V_t - \text{basal } V_t) / \text{basal } V_t \times 100\%$. No apparent discrepancy in dose dependency is seen between AVPs from two different sources. AVP from Sigma Chemical Co. (●) or Calbiochem Corp. (○).

luminal AVP. The transient hyperpolarization induced by basolateral AVP started 1 to 2 min after addition of AVP to the bath medium reached a maximum lasting only several seconds within 5 to 8 min after addition of AVP, then V_t rapidly depolarized nearly to the basal V_t level within 15 min (e.g., Fig. 3, A, B, D, and E). In the case of luminal AVP, hyperpolarization started 1 to 2 min after addition of AVP to the luminal perfusate, then maximal hyperpolarization was attained in 4 to 10 min. Subsequently, in about three-fourths of all tubules, the hyperpolarization was maintained for as long as luminal AVP was present (up to 30 min in flux studies presented later, e.g., Fig. 6 A). In one-fourth of the tubules, after the 1- to 5-min hyperpolarization V_t was partially depolarized. This depolarization was slower than that after addition of basolateral AVP, and always stabilized at levels more negative than the basal V_t (e.g., Fig. 3 A).

The V_t change induced by AVP on one side of the CCD epithelium was not influenced by the presence of AVP on the contralateral side (Fig. 3, A and B). Furthermore, as shown in Figs. 3 C-E and in Table I, the V_t change induced by basolateral AVP was abolished in the presence of basolateral ouabain, a well known inhibitor of electrogenic Na^+ and K^+ transport in the CCD (21, 22). In contrast, the V_t responses to luminal AVP and to bath isoproterenol, which is thought to alter V_t via activating adenylyl cyclase exclusively in intercalated cells (23-26), were preserved.

In the study shown in Table I, the V_t response to basolateral and luminal AVP was examined in the presence and absence of ouabain according to the protocol shown in Fig. 3 D. By adding 10 μM ouabain to the bath medium, the V_t changed rapidly from -14.0 ± 2.4 to 7.3 ± 1.4 mV ($n = 8$). In the presence of ouabain, 100 pM or 10 nM basolateral AVP (100 pM; tubules 2-5, 10 nM; tubules 6 and 7 in Table I) failed to change the V_t (mean $\Delta V_t = -0.3 \pm 0.3$ mV, not significantly different from zero by *t* test), whereas the V_t change induced by 10 nM luminal AVP (tubules 1-8) was preserved (mean $\Delta V_t = -5.3 \pm 0.9$ mV). In tubules 3-8, by removing ouabain and AVP from the bath medium and perfusate, V_t slowly became negative and stabilized at -9.4 ± 1.7 mV in 60 to 90 min. At this point, 100

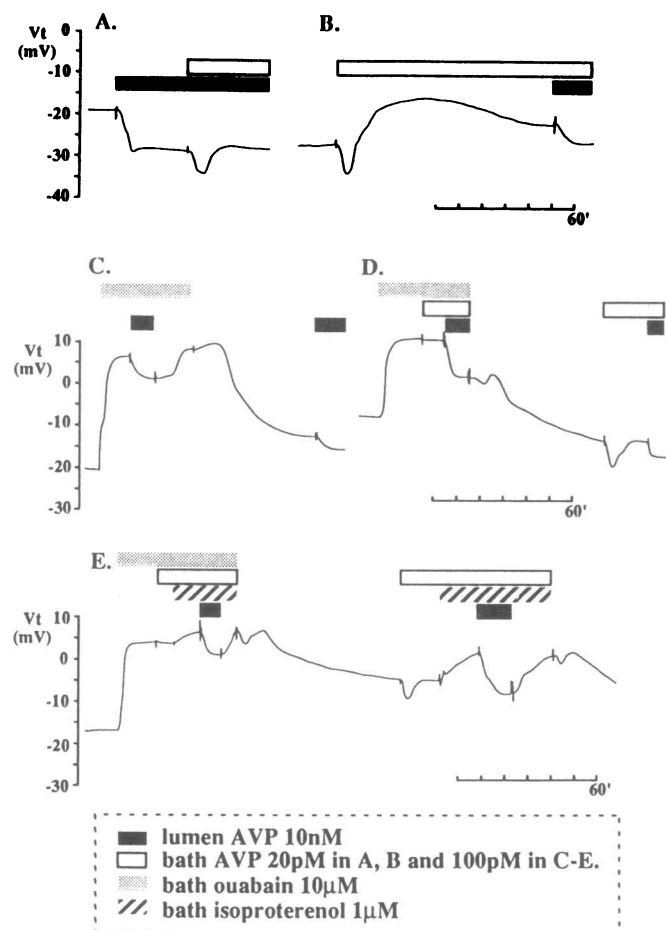


Figure 3. Comparison of the V_t change induced by basolateral AVP (20 pM in A and B, 100 pM in C-E) versus luminal AVP (10 nM). Original traces are shown. (A and B) Luminal AVP persistently hyperpolarizes V_t , whereas the hyperpolarization induced by basolateral AVP is transient (A, B, and the latter part of D and E). AVP pretreatment on one side of the tubular epithelium does not interfere with the V_t change induced by the contralateral AVP (A and B). The V_t changes induced by luminal AVP (C-E) and basolateral isoproterenol (E) are preserved in the presence of ouabain, whereas basolateral AVP fails to alter V_t when ouabain is present (D and E).

pM basolateral AVP was rechallenge in tubules 3-7. In all of these five tubules, the V_t response to basolateral AVP, i.e., transient hyperpolarization, was restored (mean $\Delta V_t = -4.0 \pm 0.9$ mV). In tubules 4-8, 10 nM luminal AVP was subsequently added, and this induced sustained hyperpolarization in all of the tubules (mean $\Delta V_t = -4.2 \pm 1.0$ mV).

Next, in a different group of tubules, net Na reabsorption and K excretion (JNa and JK , pEq/mm tubule/min) were measured using the same isotonic perfusate (Fig. 4). To enhance the changes in perfusate Na^+ or K^+ concentration during perfusion, long tubules (~ 2.0 mm) were perfused at a slow perfusion rate (~ 5 nl/min). In these experiments ($n = 6$) also, luminal AVP caused a sustained hyperpolarization of V_t (from -20.7 ± 2.9 to -34.1 ± 4.7 mV; $P < 0.01$ by paired *t* test) at a constant perfusion rate (from 5.3 ± 0.3 to 5.0 ± 0.3 nl/min; NS by paired *t* test). Interestingly, despite the evident change in V_t , JNa was stimulated only slightly (from 30.4 ± 5.5 to 32.7 ± 4.7 pEq/mm per min; $P < 0.025$ by paired *t* test) and JK did not

Table I. Differential Effect of Ouabain on Hyperpolarization of V_t by Basolateral Versus Luminal AVP

Bath ouabain		+	+	-	-
Bath AVP*		+	+	+	+
Lumen AVP		-	+	-	+
ΔV_t (mV) [†]					
Tubule	1.	NE	-8	NE	NE
	2.	-1	-7.5	NE	NE
	3.	-1	-2	-6.5	NE
	4.	0	-1	-1	-3
	5.	0	-5.5	-5	-3.5
	6.	0.5	-7	-3.5	-3
	7.	0	-6	-4	-8
	8.	NE	-5	NE	-3.5
mean \pm SE		-0.3 \pm 0.3 [‡]	-5.3 \pm 0.9	-4.0 \pm 0.9	-4.2 \pm 1.0
Scheffé multiple comparisons		P<0.001		NS	NS
		P<0.05			
			NS		

* ΔV_t (mV) was defined as: V_t at maximum hyperpolarization during exposure to basolateral luminal AVP - V_t just before each AVP addition. [‡] 100 pM in tubules 2-5, 10 nM in tubules 6 and 7. [§] Not significantly different from zero. NE, Not examined.

change significantly (from 28.5 \pm 1.7 to 29.0 \pm 1.7 pEq/mm per min; NS by paired *t* test).

To explore the mechanism of the luminal AVP-induced hyperpolarization further, we also employed acetazolamide, which is known to be an inhibitor of electrogenic H⁺ secretion in the rabbit CCD (27). In these experiments, tubules were perfused at a high perfusion rate (~ 50 nl/min), as in the dose-response and ouabain studies. In the absence of acetazolamide, the V_t was hyperpolarized by 10 nM luminal AVP from -14.9 \pm 3.1 to -21.4 \pm 3.7 mV, which recovered to -16.4 \pm 3.3 within 20 to 30 min after AVP washout (Fig. 5 and Table II). Then in the same tubules, luminal AVP was rechallenge in

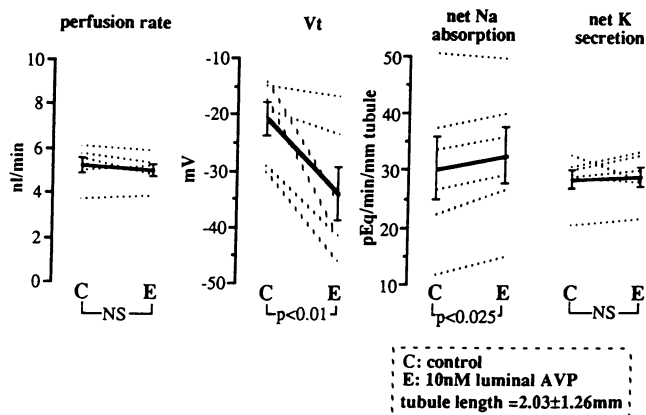


Figure 4. Effect of luminal AVP on net Na and K flux in CCD. Dotted line and solid line represent individual experiment and the mean, respectively. At a constant perfusion rate, luminal AVP hyperpolarizes V_t with negligible changes in the net Na and K fluxes. Statistical analyses were done by paired *t* test.

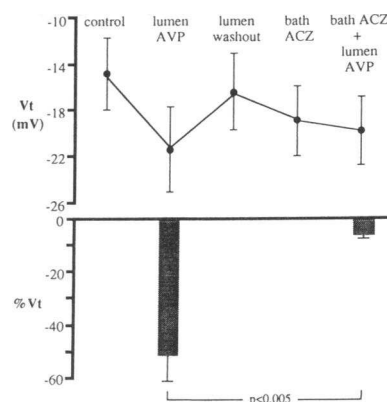


Figure 5. Effect of 200 μ M bath acetazolamide (ACZ) on 10 nM luminal AVP-induced hyperpolarization. (See Table I for individual data.) The mean V_t of five experiments is shown in the upper panel. The changes in V_t between each period were significant when compared by paired *t* test. The lower panel represents the magnitude of hyperpolariza-

tion by luminal AVP (percentage V_t) in the absence and in the presence of ACZ.

the presence of 200 μ M acetazolamide in the bath. By addition of acetazolamide, V_t was initially hyperpolarized and partially reversed, stabilizing at -18.9 \pm 3.0 mV in ~ 30 min. Subsequent luminal AVP hyperpolarized V_t only slightly to -19.8 \pm 3.0 mV. Both ΔV_t and percentage V_t changes were significantly smaller in the presence of acetazolamide (Fig. 5, Table II A).

To exclude the possibility that the attenuated hyperpolarization in response to the second luminal AVP was not due to acetazolamide, but resulted from desensitization of the tubule by the initial luminal AVP, we carried out another five time-matched experiments, in which 10 nM luminal AVP was applied twice with an interval of 40 to 60 min (Table II B). In this study, desensitization was denied since the V_t responses to the first and second luminal AVP challenges were not different. Furthermore, because the hyperpolarization induced by acetazolamide itself might have reduced the potential of the tubules to hyperpolarize further in response to luminal AVP, another procedure to hyperpolarize the V_t , i.e., luminal addition of Ba⁺⁺, was tested. Ba⁺⁺ is a well known K channel blocker and hyperpolarizes the V_t of the rabbit CCD by inhibiting apical K secretion (28). Indeed, as shown in Table II C, 2 mM luminal BaCl₂ pretreatment hyperpolarized V_t from -12.9 \pm 1.9 to -22.2 \pm 3.2 mV. Nevertheless, the V_t was further hyperpolarized to -29.4 \pm 4.8 mV by superimposing luminal AVP. Both ΔV_t and percentage V_t changes induced by luminal AVP in the presence of Ba were not significantly different from those induced by luminal AVP in untreated tubules, as shown in Table II, A and C. Thus, it was unlikely that hyperpolarization of V_t itself spoiled the V_t change induced by luminal AVP in the acetazolamide study.

Finally, the effect of luminal AVP on osmotic water permeability in CCD was investigated (Fig. 6) in the presence of an osmotic gradient between the bath medium (300 mOsmol) and the low NaCl hypotonic perfusate (150 mOsmol). To stimulate water transport, 20 pM basolateral AVP was chosen because this concentration is within the physiologic range for plasma AVP and exclusively stimulates the cyclic AMP system without considerably affecting noncyclic AMP-related signaling systems coupled to the so called V_1 type receptor (1). Also, this concentration is comparable to the plasma AVP concentration recorded in a previous human study (~ 40 pM) when nanomolar (~ 7 nM) AVP was measured in the urine (9).

Table II. Effect of Acetazolamide (ACZ) on Luminal AVP-induced Hyperpolarization

Vt of tubule	1	2	3	4	5	mean V_i	mean ΔV_i	mean % V_i
A. Effect of bath acetazolamide								
control	-15.5	-19	-9	-7	-24	-14.9±3.1		
lumen AVP 10 nM	-22	-24	-17	-11	-33	-21.4±3.7	-6.5±0.9	-50.4±10.8
lumen washout	-14	-22	-12	-8	-26	-16.4±3.3		
bath ACZ 200 μ M	-18.5	-24	-14	-11	-27	-18.9±3.0	-2.5±0.6	-19.8±6.5
bath ACZ + lumen AVP	-20	-24	-15	-11.5	-28.5	-19.8±3.0	-1.5±0.3*	-5.1±1.4*
B. Effect of repetitive lumen AVP								
control	-20	-8	-16	-7	-10	-12.2±2.5		
lumen AVP 10 nM (1st)	-27	-13	-20	-11	-15	-17.2±2.9	-5.0±0.5	-45.9±7.0
lumen washout	-16	-9	-9	-7	-9.5	-10.1±1.5		
lumen AVP 10 nM (2nd)	-20	-15	-12	-11	-15.5	-14.7±1.6	-4.6±0.6 [‡]	-49.1±8.4 [‡]
C. Effect of lumen Ba								
control	-11.5	-9	-11	-13	-20	-12.9±1.9		
lumen Ba 2 mM	-25	-17	-15	-21	-33	-22.2±3.2	-9.3±1.8 [§]	-73.8±13.7 [§]
lumen Ba + lumen AVP 10 nM	-28	-23	-21	-27	-48	-29.4±4.8	-7.2±2.0	-32.3±5.8
lumen AVP washout	-24	-19	-18	-21.5	-31	-22.7±2.3		

* $P < 0.005$ vs. lumen AVP alone. [‡] Not significantly different from first lumen AVP by paired t test. [§] $P < 0.01$ vs. bath ACZ in protocol A. ^{||} Not significantly different from lumen AVP in protocol A or those in protocol B by nonpaired t test.

Eight tubules served as controls for the effect of 20 pM basolateral AVP on V_i and L_p in the absence of luminal AVP, where V_i was transiently hyperpolarized from -19.9 ± 4.1 to -28.0 ± 5.8 mV (Fig. 6, upper panel), and L_p increased from a basal value of 5.9 ± 1.5 to a peak of $204.3 \pm 21.1 \times 10^{-7}$ cm/atm per-s (Fig. 6, lower panel). In seven other tubules, even in the presence of a NaCl concentration gradient between the perfusate and bath medium, a rapid and sustained hyperpolarization of V_i from -24.7 ± 6.9 to -34.5 ± 7.2 mV, ($n = 7$, $P < 0.005$ by paired t test) was observed upon luminal addition of 10 nM AVP at a constant perfusion rate (V_i : from 17.7 ± 0.5 to 17.0 ± 0.6 nl/min; NS by paired t test), as shown in the upper panel of Fig. 6. In these tubules, L_p was not increased (from 3.9 ± 0.8 to $5.0 \pm 1.2 \times 10^{-7}$ cm/atm per s; NS by paired t test) by luminal AVP (Fig. 6, lower panel). Though subsequent addition of 20 pM basolateral AVP further caused an intact transient hyperpolarization (Fig. 6 A; ΔV_i change = -8.9 ± 2.3 vs. -8.0 ± 2.4 mV in control experiments without luminal AVP; NS), the peak L_p induced by basolateral AVP was significantly lower in the presence of luminal AVP (Fig. 6, lower panel). Peak L_p was 134.9 ± 19.2 vs. $204.3 \pm 21.1 \times 10^{-7}$ cm/atm per s in the control ($P < 0.05$).

Discussion

This study agrees with previous in vitro microperfusion studies in which 250 μ U/ml (~ 500 pM) AVP was luminally applied to the rabbit CCD (12): luminal AVP, even at a concentration as high as 10 nM, failed to increase water permeability in contrast to basolateral AVP. However, we found for the first time that luminal AVP in the nanomolar range hyperpolarized V_i and antagonized the hydroosmotic effect of basolateral AVP. The action of luminal AVP was quite different from that of basolateral AVP, and cannot have been due to leakage of luminal AVP into the bath medium, because (a) the sustained

hyperpolarization was distinct from the transient hyperpolarization induced by basolateral AVP, (b) luminal AVP did not interfere with the V_i response to basolateral AVP, and vice versa, (c) basolateral ouabain abolished the V_i response to basolateral AVP while the V_i change induced by luminal AVP was preserved, and (d) no increase in L_p was induced by luminal AVP (basolateral AVP as low as 1 pM is enough to stimulate L_p significantly in this system [1]). A nonspecific response to the perfusate exchange per se was also denied because luminal perfusion with vehicle only or with 10 nM glucagon did not alter V_i . In addition, parathyroid hormone 100 mU/ml has been previously reported not to alter V_i in the rabbit CCD (29). Also, this V_i response appears to be localized to the CCD, because OMCDi lacked any V_i response to luminal AVP in this study, and the V_i of the medullary thick ascending limb of loop of Henle of the rat was reportedly insensitive to luminal AVP at ~ 5 nM (30).

The V_i change induced by luminal AVP seems to be unique. In the light of detailed studies of the morphological and functional subtypes of CCD cells, where a variety of ion transport pathways have been identified and found to be hormonally regulated, the origin of V_i in the CCD and the mechanism of its regulation cannot be explained simplistically without direct measurement of transport of various ions including H^+ , Cl^- , HCO_3^- , Ca^{++} , PO_4^{3-} , and Mg^{++} (31–34) in the apical and basolateral membranes of both principal and intercalated cells. However, because the principal cell is thought to participate, if not exclusively, in AVP-regulated water transport in the CCD (26, 35), the inhibition of the hydroosmotic effect of basolateral AVP by luminal AVP suggests that luminal AVP might alter V_i by directly or indirectly affecting ion transport in principal cells. Nevertheless, the V_i change induced by luminal AVP is obviously distinct from that induced by basolateral AVP, and cannot be explained only by trivial changes in net Na or K transport. In the CCD, the principal cell is considered to be

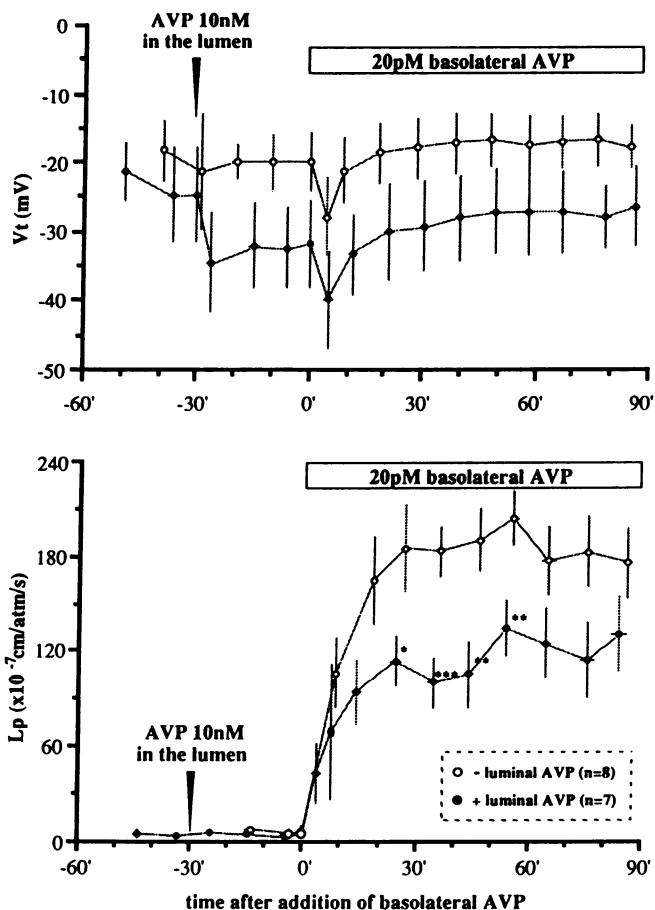


Figure 6. Effect of luminal AVP on water transport in CCD. The time courses of the mean V_t and L_p changes in control and experimental groups are shown in parallel. In the experimental group represented by solid circles, luminal AVP was added at -30 min. The upper panel shows sustained hyperpolarization by luminal AVP versus transient hyperpolarization by basolateral AVP. In the lower panel, while luminal administration of AVP itself has no significant effect on L_p , the increase in L_p induced by subsequent basolateral AVP is significantly attenuated compared with the control group during 20 to 50 min after addition of basolateral AVP ($*P < 0.05$, $**P < 0.025$, $***P < 0.005$ vs. corresponding control without luminal AVP). The peak L_p levels, defined in Methods, were also significantly different (see text).

primarily responsible for electrogenic Na^+ and K^+ transport. The driving force for transport of these cations in principal cells is provided by the basolateral ouabain-sensitive Na^+/K^+ pump (21, 22, 26). Thus, if luminal AVP hyperpolarizes V_t by modulating Na or K transport in the principal cell, the V_t change induced by luminal AVP as well as the change caused by basolateral AVP should be suppressed by ouabain (31, 36–38). However, the V_t response was preserved in the presence of ouabain (Fig. 3, C–E, Table I), in accord with the lack of considerable change in net Na or K transport (Fig. 5). Furthermore, luminal Ba^{++} did not inhibit the V_t change induced by luminal AVP (Table II C). This is also compatible with the lack of change in net K transport and the inability of ouabain to inhibit the effect of luminal AVP on V_t . Taken together, the evidence is fairly convincing that Na and K transport in principal cells is not the crucial factor for the V_t response to luminal AVP.

The effect of basolateral isoproterenol, which is thought to affect intercalated cell function preferentially (23–26), on V_t was preserved in the presence of ouabain (Fig. 3 E) in agreement with studies by others (23). Therefore, luminal AVP might hyperpolarize the V_t by modulating the transport of ions in intercalated cells. In this regard, the inhibitory effect of acetazolamide on the luminal AVP-induced hyperpolarization (Fig. 5, Table II A) is important. Electrogenic luminal acidification in the CCD, presumably via the apical H^+ pump of intercalated cells, is thought to play a role in shifting V_t in a positive direction, and acetazolamide has been found to inhibit this electrogenic acidification mechanism (27, 39). Studies by Koeppen and Helman (27) demonstrated that in the rabbit CCD, by attenuating luminal acidification, acetazolamide can shift the V_t in a negative direction in the presence of basolateral ouabain or in the absence of luminal Na^+ , both of which induce a lumen-positive V_t by abolishing Na^+ reabsorption. In our experiments, acetazolamide by itself slightly hyperpolarized V_t , and significantly inhibited the luminal AVP-induced hyperpolarization (Fig. 5, Table II A). Our additional experiments shown in Table II B and C denied that this inhibition by acetazolamide was nonspecific. Thus, luminal AVP is suggested to induce hyperpolarization by inhibiting acetazolamide-sensitive electrogenic transport. Obviously, inhibition of apical H^+ secretion is one of the most plausible mechanisms. However, this assumption needs to be confirmed by direct measurement of luminal acidification, because the effect of luminal AVP and its suppression by acetazolamide could be secondary to an alteration of intracellular pH, which is known to modify V_t (40).

If inhibition of H^+ secretion is the common mechanism responsible for hyperpolarizations induced by acetazolamide and luminal AVP, why did the former induce a smaller hyperpolarization than the latter? Although we have no direct evidence to explain this difference, one possibility is that the hyperpolarization caused by acetazolamide could be hampered by concomitant inhibition of Na^+ reabsorption. Both Stoner et al. (39), and Koeppen and Helman (27) observed that the effect of acetazolamide in increasing the negativity of V_t became overt if Na^+ reabsorption was inhibited previously by ouabain, a Na-free perfusate, or luminal amiloride. In contrast, because luminal AVP did not inhibit Na^+ reabsorption (Fig. 4), the hyperpolarization induced by luminal AVP would not be mitigated.

Also, in some studies (27, 41), basolateral ouabain per se has been found to suppress luminal acidification in the CCD. If this suppression is due to the same mechanism as that induced by acetazolamide, ouabain might also suppress luminal AVP-induced hyperpolarization. However, this was not obvious in our study (Table I), probably because of a difference in the mechanism or potency of these two agents in inhibiting acidification. Indeed Koeppen and Helman did not observe inhibition of acidification by ouabain in the absence of extracellular Na and K, whereas that induced by acetazolamide was independent of these extracellular cations (27).

The mechanism of inhibition of the hydroosmotic effect of basolateral AVP by luminal AVP has also been unexplored. At least one previous study, in which no AVP-sensitive adenylyl cyclase was detected in the luminal membrane fraction of the bovine collecting duct, appears to argue against the possibility that the inhibition is related to an increase in cyclic AMP by luminal AVP (42). As mentioned earlier, nanomolar AVP is capable of autoinhibiting the hydroosmotic effect through activation of protein kinase C, elevation of the intracellular free

Ca⁺⁺ concentration, or stimulation of prostaglandin synthesis (1). If certain AVP receptors coupled to these machineries reside in the apical membrane, luminal AVP might inhibit the hydroosmotic effect of basolateral AVP. In this regard, it is noteworthy that apical AVP has been found to stimulate prostaglandin synthesis in canine CCD cells in primary culture (15). However, all three of these mechanisms have been found to depolarize V_i when activated. The protein kinase C activators phorbol myristate acetate and dioctanoylglycerol, the Ca ionophores A23187 and ionomycin, and prostaglandin E₂ rapidly depolarize the V_i of CCD when applied from the basolateral side (5–7, 18, and our unpublished data). In addition, all of these three signals are known to inhibit transepithelial Na reabsorption (5–7) in the CCD. Thus, it seems unlikely that these autoregulation mechanisms are employed by luminal AVP to inhibit water transport without either depolarizing the V_i or inhibiting Na transport. However, we cannot rule out the possibility that luminal AVP selectively or locally activates these signals in the apical membrane or subapical cytoplasm, modulating cell functions in fashions different from those mentioned above.

The inhibition of the hydroosmotic effect by luminal AVP may be secondary to a change in electrolyte transport. The change in electrolyte transport underlying the luminal AVP-induced sustained hyperpolarization would alter the electrolyte composition or pH of the luminal perfusate as well as that of the intracellular fluid. Previous studies on amphibian urinary bladder and mammalian CCD (40, 43–45) have shown that the hydroosmotic effect of basolateral AVP is sensitive to alteration in extracellular and/or intracellular pH and electrolyte composition. Such inhibitory mechanisms may function in either of the two cell types in the CCD, because the osmotic water permeability of intercalated cells as well as that of principal cells is reported to be increased in response to basolateral AVP (26, 35).

Finally, it also remains to be explored how AVP interacts with the luminal surface of the CCD. Although regulation of tubular epithelial functions by hormones in the lumen (urine) has been largely unexplored, a few hormones or biologically active substances are known or are suggested to act from the apical side of the renal tubule. For example, dopamine receptors have been found on the apical membrane of the proximal tubule (46). In the rabbit medullary thick ascending limb of loop of Henle, luminal prostaglandin E₂ has been found to depolarize V_i (47). Also, it has been found that an apical G protein linked to an ion channel exits in the rat medullary collecting duct (48). In the light of a previous study by Ando et al. (1), the higher threshold concentration for the action of luminal AVP compared with that of basolateral AVP and the antagonistic action of luminal AVP on water transport imply that a V₁-type receptor might reside on the apical membrane. However, studies should be undertaken with caution before classifying the luminal receptor for AVP, if present, as either the V₁ or V₂ type, because the interaction of AVP and CCD on the luminal surface may not be mediated by previously defined AVP receptors for signal transduction. Also, cross-reaction of AVP and oxytocin receptors should be considered (49). Furthermore, an early in vivo clearance study suggested that AVP filtered or excreted into urine might interact with catabolic enzymes on the luminal surface of the distal nephron segment (50). Such an interaction of the apical membrane of CCD cells and AVP for digestion might alter CCD functions secondarily.

Regardless of the mechanisms underlying the luminal action of AVP, an intriguing hypothesis is deducible. The concentration of urinary AVP would not be as high as the nanomolar range to exert its effect in the CCD under euhydration or mild dehydration (9–11). However, among the numerous factors and conditions that affect urine production in vivo, some might attenuate the antidiuretic action of basolateral AVP by increasing the urinary excretion of AVP. This inference seems reasonable to explain a paradox between the release of AVP and the local action of AVP in the kidney. For example, prostaglandins, kinins, and catecholamines are well known inhibitors of the antidiuretic action of AVP in the kidney (17, 19, 25, 47). However, they also stimulate the release of AVP from the neurohypophysis (8). Thus, under circumstances where these “renal” AVP antagonists are systemically increased, e.g., physical stress, excessive water reabsorption in the CCD due to elevated circulating AVP may be mitigated not only by the actions of these antagonists in the kidney, but also, in part, by the drastically increased AVP in the tubular lumen. In SIADH (syndrome of inappropriate secretion of antidiuretic hormone), a high luminal AVP concentration might also play a role in facilitating urine output, despite high levels of plasma AVP.

In conclusion, this study has demonstrated that luminal (urinary) AVP in the CCD modulates water and transepithelial voltage distinctly from basolateral (plasma) AVP. It is suggested that urine production is modulated by AVP at the CCD epithelium amphilaterally in vivo. The mechanisms as well as the physiological significance of these actions of luminal AVP awaits further elucidation.

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