Feedback Inhibition of Cyclic Adenosine Monophosphate–stimulated Na^+ Transport in the Rabbit Cortical Collecting Duct via Na^+-dependent Basolateral Ca^{++} Entry

Matthew D. Breyer
with the technical assistance of **Deidrienne Fredin**
Department of Veterans Affairs Medical Center, Nashville, and Division of Nephrology and Department of Medicine, Vanderbilt University, Nashville, Tennessee 37212

Abstract

Arginine vasopressin (AVP) transiently stimulates Na^+ transport in the rabbit cortical collecting duct (CCD). However, the sustained effect of both AVP and its putative second messenger, cyclic adenosine monophosphate (cAMP), on Na^+ transport in the rabbit CCD is inhibitory. Because maneuvers that increase $[Ca^{++}]_i$ inhibit Na^+ transport, the effects of AVP and cell-permeable cAMP analogues, on $[Ca^{++}]_i$ were investigated in fura-2–loaded in vitro microperfused rabbit CCDs. Low-dose AVP (23–230 pM) selectively stimulated Ca^{++} influx, whereas 23 nM AVP additionally released calcium from intracellular stores. 8-chlorophenylthio-cAMP (8CPTcAMP) and 8-bromo-cAMP (8-Br-cAMP) also increased CCD $[Ca^{++}]_i$. The 8CPTcAMP-stimulated $[Ca^{++}]_i$ increase was totally dependent on basolateral $[Ca^{++}]$. In the absence of cAMP, peritubular Na^+ removal produced a marked increase in $[Ca^{++}]_i$, which was also dependent on bath $[Ca^{++}]$, suggesting the existence of basolateral Na^+/Ca^{++} exchange. Luminal Na^+ removal in the absence of cAMP did not alter CCD $[Ca^{++}]_i$, but it completely blocked the cAMP-stimulated $[Ca^{++}]_i$ increase. Thus the cAMP-dependent Ca^{++} increase is totally dependent on both luminal Na^+ and basolateral Ca^{++}, suggesting the $[Ca^{++}]_i$ increase is secondary to cAMP effects on luminal Na^+ entry and its coupling to basolateral Na^+/Ca^{++} exchange. 8CPTcAMP inhibits lumen-to-bath ^{22}Na flux $[J_{Na}(l\text{-}b)]$ in CCDs bathed in a normal Ca^{++} bath (2.4 mM). However, when bath Ca^{++} was lowered to 100 nM, a maneuver that also blocks the 8CPTcAMP $[Ca^{++}]_i$ increase, 8CPTcAMP stimulated, rather than inhibited $J_{Na}(l\text{-}b)$. These results suggest that cAMP formation initially stimulates CCD Na^+ transport, and that increased apical Na^+ entry secondarily activates basolateral Ca^{++} entry. The cAMP-dependent $[Ca^{++}]_i$ increase leads to inhibition Na^+ transport in the rabbit CCD. (*J. Clin. Invest.* 1991. 88:1502–1510.) Key words: calcium • collecting duct • cyclic AMP • exchanger • sodium • transport

Portions of this work were presented at the May 1990 meeting of the American Federation of Clinical Research, Washington, DC, and the December 1990 meeting of the American Society of Nephrology, Washington, DC.

Address reprint requests to Dr. Breyer, F425-ACRE Building, Department of Veterans Affairs Medical Center, Nashville, TN 37212.

Received for publication 12 July 1990 and in revised form 29 April 1991.

The Journal of Clinical Investigation, Inc.
Volume 88, November 1991, 1502–1510

Introduction

Arginine vasopressin (AVP)[1] is a major regulator of renal salt and water excretion (1, 2). Through its effects on the collecting duct, AVP increases renal water reabsorption and modulates sodium absorption (3–5). Although most of the transport effects of AVP have been attributed to increased 3'5' cyclic adenosine monophosphate (cAMP) generation (6–8), there is also evidence that intracellular Ca^{++} concentration ($[Ca^{++}]_i$) plays an important role in AVP's action (9–15). Nanomolar AVP has been shown to increase $[Ca^{++}]_i$ in isolated collecting duct cells (13–15). Burnatowska-Hledin et al. (15) have found 10^{-6} M AVP stimulates inositol phosphate formation in immunodissected rabbit cortical collecting duct (CCD) cells and have attributed the Ca^{++} increase to phosphatidylinositol bisphosphate hydrolysis (15). However, studies by Ando et al. (13) suggest that picomolar concentrations of AVP may not significantly activate phosphatidylinositol bisphosphate hydrolysis in the isolated perfused rabbit CCD. This raises into question the physiologic role of the AVP-stimulated $[Ca^{++}]_i$ increase (14), and suggests a singular role for cAMP signaling in mediating AVP's effects in the CCD. Nevertheless, maneuvers that increase $[Ca^{++}]_i$ in the collecting duct have potent inhibitory effects on sodium transport (11). In the rabbit CCD both AVP and cAMP inhibit sodium transport (4, 5, 16). The purpose of the present studies was to examine the role of $[Ca^{++}]_i$ in mediating the cAMP-dependent effects of AVP on sodium transport in the rabbit CCD.

Methods

General microperfusion methods

In vitro microperfusion of isolated CCDs was performed as previously described (13). Female New Zealand white rabbits, weighing 1.5–2.5 kg, were anesthetized with an i.m. injection of xylazine (10 mg/kg) and ketamine (40 mg/kg) and killed by decapitation. The left kidney was quickly removed, and 1–2-mm coronal slices were placed into chilled (4°C) dissection medium for freehand microdissection. Microdissected CCDs (1.0–2.2 mm) were transferred to a 0.150-ml, temperature-regulated perfusion chamber. CCDs were cannulated and perfused with concentric micropipettes.

Bath solutions were continuously exchanged. Flow rate was maintained between 0.5 and 2.5 ml/min by a perfusion pump (model 341B,

1. *Abbreviations used in this paper:* AVP, arginine vasopressin; CCD, cortical collecting duct; CPT, -chlorophenylthio.

SAGE Instruments, Orion Research, Inc., Boston, MA). All experiments were performed at 37°C. The bath solution was warmed either by preheating with a water-jacketed line, or using a thermostatically controlled perfusion chamber. Both techniques have been previously described (13).

The composition of standard bath medium, dissection medium, and perfusate was as follows (in millimolar): NaCl, 105; $NaHCO_3$, 25; Na acetate, 10; Na_2HPO_4, 2.3; KCl, 5; $CaCl_2$, 2.4; $MgSO_4$, 1.0 glucose, 8.3; and alanine, 5 (osmolality 300 mosmol). Sodium-free solutions were made by substituting sodium salts with tetramethylammonium salts. HCO_3^- was generated by bubbling tetramethylammonium-OH with 5% CO_2/95% O_2. Ca^{++}-free bath solutions were identical to the standard bath solution except for the omission of calcium and the addition of 2 mM EGTA. The 100 nM calcium bath was identical to the calcium-free bath except for the addition of 1.5 mM Ca^{++}.

Transepithelial voltage (V_t) was measured by a Ringer's-agarose bridge connected to the perfusion pipette and a calomel electrode. A similar bridge connected the bath to another calomel electrode and completed the circuit. V_t (mV) was measured with an electrometer (model KS-700, World Precision Instruments, New Haven, CT) and continuously recorded on a strip-chart recorder (Primeline model R-02, Soltec Co., Sun Valley, CA). In some experiments the voltage output was digitized into the computerized data file using the deltascan reference cell quantum counting channel as an input channel.

Measurement of $[Ca^{++}]_i$ in isolated perfused CCDs

Tubules were loaded with fura-2 (17) by bathing in 2.5 μM of the acetoxy-methyl ester of fura-2 (fura 2-AM) for 45 min at 30°C (flow rate 0.5 ml/min). After tubules were loaded, the bath temperature was increased to 37°C, the flow rate increased to 2.5 ml/min, and CCDs were allowed to equilibrate for 20–30 min. Intracellular fura-2 fluorescence intensity was measured using continuous rapidly alternating excitation (20 ms per reading) from dual monochromaters set at 340 and 380 nm, respectively (Deltascan, Photon Technology International, New Brunswick, NJ). The monochromater output was coupled to the inverted microscope using a 400-nm dichroic mirror and a 100× lens (Nikon fluor oil immersion, N.A. = 1.3). Fluorescent emission intensity was measured by photon counting through a 435-nm long-pass filter (Nikon). Before loading with fura-2, CCD autofluorescence and background light were measured (< 10% of fluorescent emission in fura-2–loaded tubules) and this value was continuously subtracted from all measurements. The corrected emission intensity ratio, using 340- and 380-nm excitation (340/380 ratio, R), was monitored continuously.

After fura-2 loading and equilibration, a baseline reading of 100–200 s was made in standard bath medium. At the end of each experiment an in situ calibration of $[Ca^{++}]_i$ was performed. The bath medium was changed to a Ca^{++}- and Mg^{++}-free isotonic solution containing EGTA and 10 μM 4Br-A23187. After a stable 340/380 ratio (minimum ratio, Rmin) was achieved the bath was changed back to the 2.4 mM Ca^{++} medium plus 10 μM 4Br-A23187 and the ratio was again allowed to stabilize (maximum ratio, Rmax).

In most experiments tubule perfusion rate was maintained at 20 nl/min or more by hydrostatic pressure. In experiments where perfusate exchanges were performed, perfusion flow was driven by pressurized air (10 psi) attached to one of four different perfusate reservoirs. In these studies continuous exchange of the perfusate was maintained during the 20-min equilibration period and throughout the experiment (at an approximate rate of 1–2 ml/min) through a concentric PE-50 line opening near the tip of the perfusion pipette. Forward perfusate flow through the tubule was ~ 100 nl/min. The remaining volume of the 1 ml/min perfusate flow was collected as waste into a trap from the back or the perfusion pipette. Preliminary studies used the fluorophore 6-methoxy-N-(3-sulfopropyl)-quinolonium, 10 mM, in the lumen to measure wash-in and wash-out kinetics (18). This showed > 90% complete perfusate exchange was accomplished in < 10 s, and perfusate exchange was complete within 20 s.

Measurement of sodium transport (J_{Na}) in isolated perfused CCDs

Experiments examining CCD sodium transport (J_{Na}) used 25 μCi/ml luminal ^{22}Na and 100 μCi/ml of [^{3}H]inulin as a volume marker. Timed perfusate collections were made into a constriction pipette of known volume (29 or 40 nl). After 90 min of equilibration four collections were made for calculation of basal J_{Na}. In the experimental period 0.1 mM 8-chlorophenylthio-cAMP (8-CPTcAMP) was added to the bath and four additional timed collections were made. In all experiments a final period of J_{Na} measurements was made after 20 min pretreatment with 10^{-4} M peritubular ouabain. Ouabain inhibits net J_{Na} close to zero (19), and thus the residual lumen-to-bath (l-b) J_{Na} is passive and comparable to bath-to-lumen (b-l) J_{Na}.

Calculations

Intracellular Ca^{++} concentration. Intracellular Ca^{++} concentration ($[Ca^{++}]_i$) was calculated by: $[Ca^{++}]_i = K_d$ (R-Rmin)/(Rmax-R) (380 min/380 max) assuming that the K_d value for the fura-2–Ca^{++} complex is 224 nM at 37°C (17).

Calculation of calcium activity in Ca^{++}/EGTA buffers. The extracellular calcium concentration was set by mixing millimolar amounts of calcium and EGTA in proportions that yielded the desired free calcium activity as determined by a computerized activity table (20). For these studies a mixture of 1.5 mM calcium and 2 mM EGTA yields a free calcium activity of ~ 100 nM.

^{22}Na flux. J_{Na}(l-b) was calculated from the rate of disappearance of tracer from the perfusate, using the following equation: $J_{Na}(l\text{-}b) = (1 - VoC^*o/ViC^*i) \times 145 \times L^{-1} = pmol \cdot mm^{-1} \cdot min^{-1}$, where C*i and C*o are perfused and collected fluid concentrations of ^{22}Na (cpm/nl), assuming constant specific activity along the tubule length, and Vi is the perfusion rate and Vo the collection rate in terms of nl/min. Vi was calculated using the inulin counts according to the relationship: Vi = (c*o/c*i) Vo, where c*o and c*i represent the cpm/nl of inulin per nanoliter of collectate and perfusate, respectively. Perfusion rates were adjusted (2–4 nl/min) so that only a small amount of perfusate ^{22}Na was lost along the tubule, ensuring relative axial uniformity of tracer specific activity. In order to facilitate comparison between control and post-cAMP periods, in high and low Ca^{++} bath groups, data for each tubule were normalized to the mean for that group. The J_{Na} for a given collection was multiplied by the basal J_{Na} for that tubule divided by the mean basal J_{Na} for the group.

Statistics

Data are presented as mean±standard error (SE) and statistical analyses were made using paired Student's *t* test or one-way analysis of variance (ANOVA) where appropriate. Differences of $P < 0.05$ were considered statistically significant.

Reagents

AVP, dDAVP, 8-CPTcAMP, and EGTA were purchased from Sigma Chemical Co., St. Louis, MO. AVP and dDAVP were also purchased from Peninsula Laboratories, Belmont, CA. Dimethylsulfoxide (DMSO) was purchased from Serva, Westbury, NY. SPQ, fura-2-AM, and 4Br-A23187 were purchased from Molecular Probes, Eugene, OR. ^{22}Na and [^{3}H]inulin were obtained from New England Nuclear, Boston, MA. All other reagents were purchased from Sigma Chemical Co.

Results

Effect of cell-permeant cAMP analogues on CCD $[Ca^{++}]_i$. 8CPTcAMP (0.1, 0.2, or 1 mM) increased CCD $[Ca^{++}]_i$ (Table I, Fig. 1 *A*). Within 30 s of addition of 0.2 mM 8CPTcAMP $[Ca^{++}]_i$ began to increase and peak $[Ca^{++}]_i$ occurred 211±52 s after 8CPTcAMP addition, increasing from a mean resting calcium of 149.6±45 to 293.4±90 nM ($n = 8$, $P < 0.005$). $[Ca^{++}]_i$

Table I. Effect of Agents That Increase CCD cAMP on $[Ca^{++}]_i$

Bath $[Ca^{++}]$	2.4 mM Basal	2.4 mM Agonist stimulated (*n*)	<1 nM Basal	<1 nM Agonist stimulated (*n*)
Agonist	Intracellular $[Ca^{2+}]$, *nM*			
AVP				
2×10^{-11} M	90±4	125±10* (9)	—	—
2×10^{-10} M	74±19	136±19* (12)	45±5	62±9 (4)
2×10^{-8} M	74±19	444±96* (12)	45±5	123±10* (4)
dDAVP				
10^{-10} M	99±18	136±36* (9)	—	—
10^{-8} M	99±18	272±61* (9)	81±40	111±42* (3)
Isoproterenol	92±21	96±20 (5)	—	—
Forskolin	80±16	102±12* (6)	—	—
8 Br cAMP	183±87	409±229* (4)	—	—
8 CPTcAMP				
1×10^{-4} M	78±11	160±3* (3)	—	—
2×10^{-4} M	149±45	293±90* (8)	61±12	72±8 (5)
1×10^{-3} M	89±43.4	200±70* (4)	—	—

* $P < 0.05$ vs. basal.

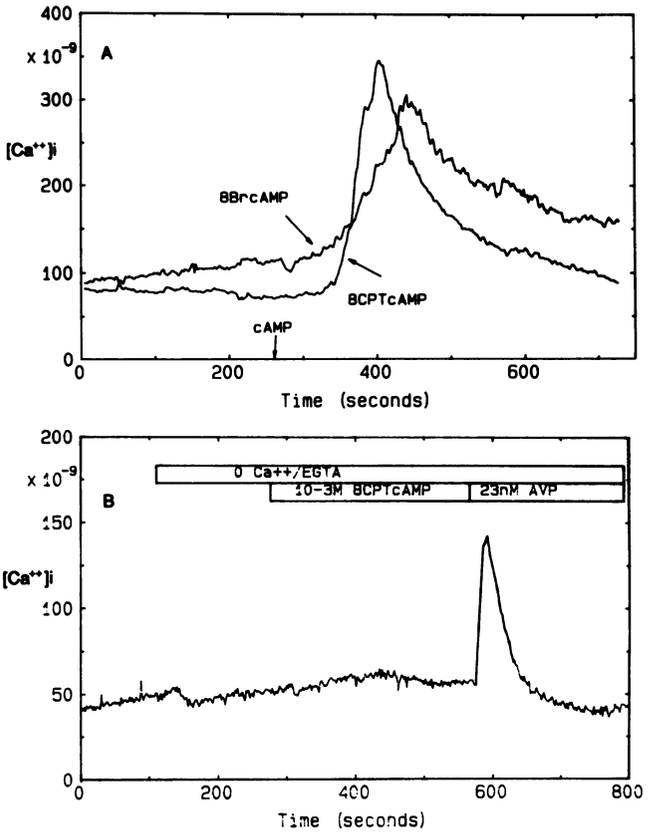

Figure 1. Cell-permeable cAMP analogues increase $[Ca^{++}]_i$ influx. $[Ca^{++}]_i$ is plotted on the vertical axis in nanomolars. (*A*) Effect of either bath 8CPTcAMP (0.2 mM) or 8-Br-cAMP (0.2 mM) on $[Ca^{++}]_i$. Analogues were added at the time denoted by the arrow labeled *cAMP*. (*B*) 1 mM 8CPTcAMP does not increase $[Ca^{++}]_i$ in the absence of extracellular Ca^{++}, but 23 nM AVP still releases intracellular Ca^{++}.

subsequently returned to baseline within 500–600 s. Similar results were obtained with a second cell-permeant cAMP analogue, 8-Br-cAMP. In four separate experiments, addition of 0.2 mM 8-Br-cAMP increased $[Ca^{++}]_i$ from 183±87 to 409±229 nM (Fig. 1 *A*, Table I). Thus, as opposed to studies in the rat inner medullary collecting duct (12), exogenous cell-permeable cAMP analogues increase $[Ca^{++}]_i$ in the rabbit CCD.

Source of the 8CPTcAMP-stimulated increase in CCD $[Ca^{++}]_i$. The effect of 8CPTcAMP on CCD $[Ca^{++}]_i$ was studied in the absence of extracellular Ca^{++} (Fig. 1 *B*). 1 mM 8CPTcAMP had no effect on $[Ca^{++}]_i$ in CCDs bathed and perfused with 0 Ca^{++} buffers (basal 61±12.2 to 1 mM 8CPTcAMP 72±8.3, $n = 5$, P = ns). As a positive control, 23 nM AVP significantly increased $[Ca^{++}]_i$ in these same CCDs. Thus the cAMP-stimulated increase in $[Ca^{++}]_i$ is dependent upon extracellular Ca^{++} and not upon the release of Ca^{++} from intracellular stores. Conversely, 23 nM AVP is capable of releasing calcium from intracellular stores, by a mechanism that is clearly distinct from that activated by increased cell cAMP.

Effect of AVP on CCD $[Ca^{++}]_i$. 23 pM, 230 pM, and 23 nM AVP all significantly increased intracellular calcium in the rabbit CCD (Fig. 2, Table I).[2] Like cAMP analogues, 23 or 230 pM AVP significantly increased intracellular Ca^{++}. In the absence of extracellular Ca^{++} 230 pM AVP failed to increase $[Ca^{++}]_i$, showing that, like cAMP, this effect was due to calcium influx. In contrast, 23 nM AVP significantly increased intracellular Ca^{++} both in the presence and absence of extracellular Ca^{++}. In the presence of extracellular Ca^{++} 23 nM AVP produced an abrupt transient increase in $[Ca^{++}]_i$, followed by a lower but sustained elevation in $[Ca^{++}]_i$. When extracellular Ca^{++} was removed the transient increase was still present but the sustained elevation in $[Ca^{++}]_i$ was no longer seen. Similar dose-dependent results were obtained with the putative V_2-selective analogue dDAVP (21) (Table I).

Effect of forskolin (FSK) on CCD $[Ca^{++}]_i$. To determine whether stimulation of endogenous cAMP generation also increased $[Ca^{++}]_i$ in the CCD, we tested the effect of 100 μM FSK on CCD $[Ca^{++}]_i$. This concentration of FSK produces maximal increase in osmotic water permeability (22). FSK significantly increased $[Ca^{++}]_i$ (Fig. 3, Table I: basal $[Ca^{++}]_i$ 80±16 nM to +FSK 102±12 nM, $P < 0.05$, $n = 6$).

Effect of isoproterenol on CCD $[Ca^{++}]_i$. Because isoproterenol significantly increases CCD cAMP generation (23, 24), we tested whether isoproterenol also increased $[Ca^{++}]_i$ in the CCD. In contrast to the effects of AVP, dDAVP, FSK, or cell-permeable cAMP analogues, no significant effect of 0.1 mM isoproterenol on intracellular Ca^{++} was seen (Table I).

Temporal relationship between intracellular voltage and $[Ca^{++}]_i$. Simultaneous digital recordings of transepithelial voltage (V_t) and $[Ca^{++}]_i$ were obtained (Fig. 4). 8CPTcAMP produced an initial negative deflection in V_t followed by a second phase in which V_t became more positive. The 8CPTcAMP-in-

2. Although our previous (13) studies had not found a significant effect of 23 pM AVP on $[Ca^{++}]_i$ in the CCD, those studies were performed in the absence of luminal calcium. In the present studies luminal calcium was present unless otherwise stated.

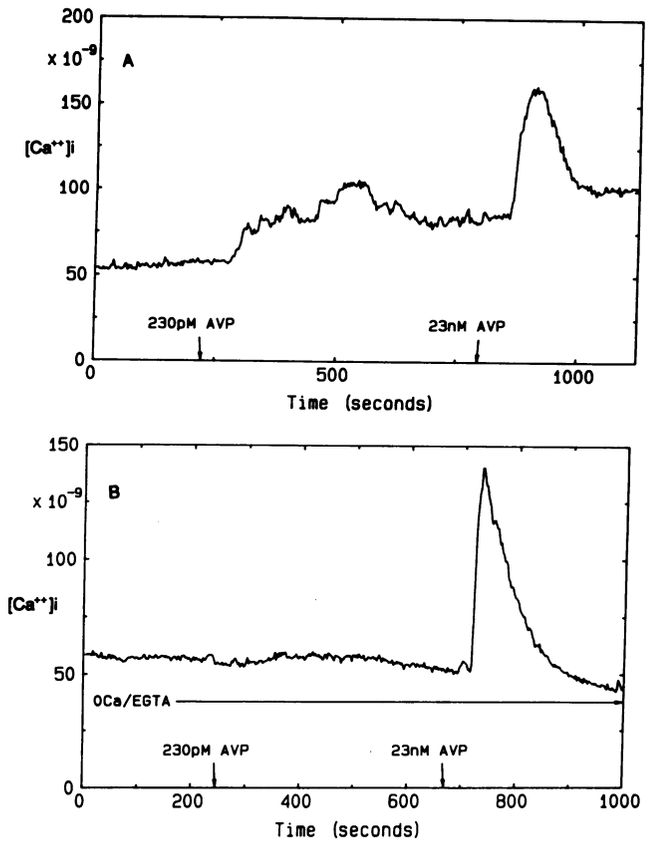

Figure 2. Dose-dependent effect of AVP on CCD $[Ca^{++}]_i$. $[Ca^{++}]_i$ is plotted on the left vertical axis in nanomolars. (*A*) Effects of 230 pM (100 μU/ml) and 23 nM (10 mU/ml) AVP in the presence of extracellular Ca^{++}. (*B*) Similar experiment conducted in the absence of extracellular Ca^{++} (0 Ca^{++}/EGTA buffers).

duced $[Ca^{++}]_i$ increase occurred either simultaneously or just slightly after the lumen-negative voltage deflection and preceded the lumen-positive voltage deflection.

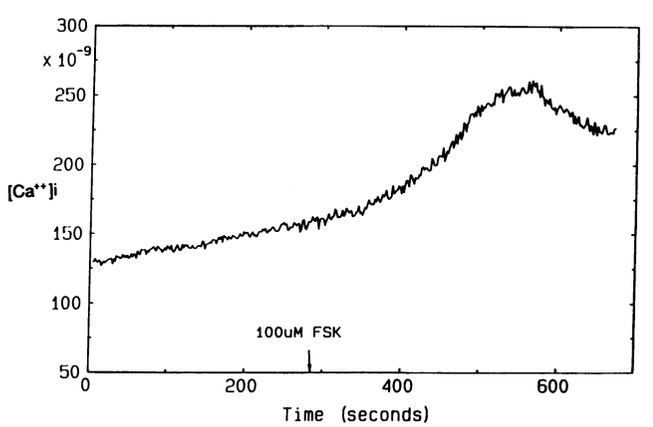

Figure 3. FSK increases CCD $[Ca^{++}]_i$. The effect of 100 μM forskolin on $[Ca^{++}]_i$ in the CCD. FSK was administered from the bath and at the labeled arrow. $[Ca^{++}]_i$ is plotted on the left vertical axis in nanomolars.

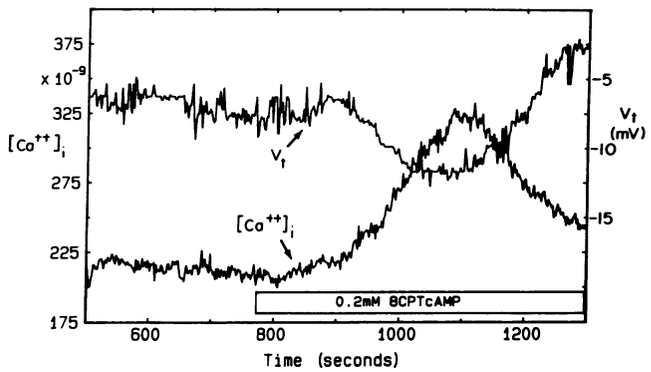

Figure 4. The temporal relationship between the 8CPTcAMP-induced change in $[Ca^{++}]_i$ and transepithelial voltage. The effect of 0.2 mM 8CPTcAMP on simultaneous recordings of $[Ca^{++}]_i$ and transepithelial voltage is shown. $[Ca^{++}]_i$ is plotted on the left vertical axis in nanomolars.

Effect of luminal sodium on 8CPTcAMP-induced Ca^{++} influx. Since the lumen-negative V_t in the CCD reflects electrogenic Na^+ transport (25), we tested whether 8CPTcAMP induced Ca^{++} influx was dependent on luminal Na^+ (Fig. 5, Table II). Before 8CPTcAMP treatment, luminal Na^+ removal had no effect on $[Ca^{++}]_i$, and readdition produced a slight but statistically insignificant increase in intracellular Ca^{++} (from 155±55.4 to 185±47.8, P = ns). Importantly, when 0.2 mM 8CPTcAMP was added to the bath in the absence of luminal Na^+, no increase in $[Ca^{++}]_i$ was observed (from 162±46.3 to 157±48, P = ns). However, when luminal Na^+ was readded to the 8CPTcAMP-treated CCD, there was a 23-fold greater increase in $[Ca^{++}]_i$ (from 157±48 to 855±251, $P < 0.05$, ANOVA) than when luminal sodium was readded before 8CPTcAMP treatment.

To determine whether the effect of cAMP and luminal Na^+ readdition was dependent on Na^+ and not cell depolarization (26) which might activate voltage-operated calcium channels

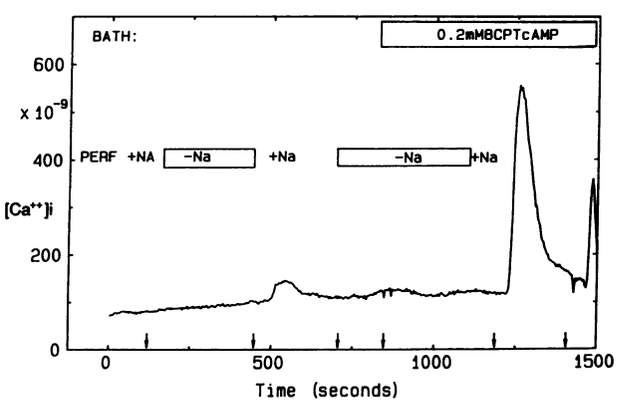

Figure 5. The effect of 8CPTcAMP on $[Ca^{++}]_i$ is dependent on luminal Na^+. $[Ca^{++}]_i$ is plotted on the left vertical axis in nanomolars. The tracing of intracellular Ca^{++} shows a small effect of perfusate (*PERF*) Na^+ replacement with TMA^+ (*−Na*) and readdition, on $[Ca^{++}]_i$ before cAMP treatment. Luminal Na^+ was again removed. This blocked the 0.2 mM 8CPTcAMP-stimulated $[Ca^{++}]_i$ increase over a 350-s exposure. Subsequent readdition of perfusate sodium produced marked increase in CCD $[Ca^{++}]_i$.

Table II. Effect of Na^+ and Ca^{++} removal on $[Ca^{++}]_i$ in Untreated and 8CPTcAMP-treated CCDs

Maneuver	$\Delta[Ca^{++}]_i$, nM	n
Untreated		
Basolateral Na^+ removal (+ bath Ca^{++})	440±114*	5
Basolateral Na^+ removal (− bath Ca^{++})	67±34	7
Luminal Na^+ removal	9±5	4
Luminal Na^+ readdition	30±15	4
+8-CPT-cAMP		
8CPTcAMP (control)	147±48*	8
8CPTcAMP (− luminal Ca^{++})	55.5±13*§	8
8CPTcAMP (100 nM bath Ca^{++})	5±3‡	5
8CPTcAMP (− luminal Na^+)	−6±5‡	4
8CPTcAMP luminal Na^+ readdition	698±212*¶	4

* $P < 0.05$ vs. basal.
‡ $P < 0.05$ vs. 8CPTcAMP (control).
§ $P < 0.05$ vs. basolateral Ca^{++} removal.
¶ $P < 0.05$ vs. Na^+ readdition without 8CPTcAMP.

(27), we depolarized the CCD by raising bath K^+ from 5 to 20 mM. This had no effect on $[Ca^{++}]_i$ (basal 179.5±56 to high K^+ 163±59, $n = 7$). Thus the capacity of Na^+ readdition to raise $[Ca^{++}]_i$ is dependent on luminal Na^+ per se and not depolarization of the CCD cell.

Polarity of 8CPTcAMP-stimulated Ca^{++} influx. We examined the effect of 0.2–1 mM 8CPTcAMP on $[Ca^{++}]_i$ after either peritubular or luminal Ca^{++} was selectively removed (0 Ca^{++}/2 mM EGTA buffer) (Table II). In CCDs perfused with Ca^{++}-free buffer, 0.2 mM 8CPTcAMP significantly increased $[Ca^{++}]_i$ (basal 135±34.5 to +8CPTcAMP 190.5±39.1, $n = 8$, $P < 0.05$). However, when peritubular Ca^{++} was selectively removed, 8CPTcAMP failed to increase $[Ca^{++}]_i$ (Table II). Similarly lowering peritubular $[Ca^{++}]$ to 100 nM prevented the effect of 8CPTcAMP pretreatment and luminal Na^+ readdition on $[Ca^{++}]_i$ (Fig. 6). Readdition of 2.4 mM bath Ca^{++} to 8CPTcAMP-treated CCDs produced a prompt increase in $[Ca^{++}]_i$ above baseline. Thus, 8CPTcAMP significantly increases basolateral Ca^{++} influx, but only when Na^+ is present in the lumen.

Effect of bath sodium removal on CCD $[Ca^{++}]_i$. The replacement of peritubular Na^+ with TMA^+ produced a rapid and reversible increase in intracellular $[Ca^{++}]_i$ (basal, $+Na^+$ 202±57 nM to $-Na^+$ 642±171 nM, $n = 5$ CCDs, $P < 0.01$, see Table II). In the absence of basolateral Ca^{++}, bath Na^+ removal failed to significantly increase $[Ca^{++}]_i$ (from 53±13 to 120.4±43, P = ns, $n = 7$), suggesting that bath Na^+ removal increases basolateral Ca^{++} influx (Fig. 7, Table II). Fig. 7 shows that luminal Na^+ removal also blunts the $[Ca^{++}]_i$ increase seen after bath Na^+ is removed. Bath sodium removal markedly increases $[Ca^{++}]_i$ only after bath Ca^{++} is restored but addition of luminal Na^+ causes a further increase in $[Ca^{++}]_i$, that is reversed by readding bath sodium. Basolateral Ca^{++} influx is increased by luminal Na^+ readdition and decreased by bath sodium readdition.

Role of bath Ca^{++} in cAMP-dependent inhibition of sodium transport. 0.1 mM 8CPTcAMP inhibits sodium transport in the rabbit CCD (16, 28). The present studies characterized the time course of this effect and determined its dependence on bath $[Ca^{++}]$ (Fig. 8). In a 2.4 mM Ca^{++} bath, 0.1 mM 8CPTcAMP caused a statistically insignificant increase in J_{Na}(l-b) over the first 20 min and subsequently J_{Na}(l-b) fell from a basal of 30.3±1.0 to 25.4±2 pmol · mm^{-1} · min^{-1} ($n = 7$, $P < 0.05$, ANOVA). In a 100 nM Ca^{++} bath, (a condition which blocks the 8CPTcAMP-induced $[Ca^{++}]_i$ increase) 0.1 mM 8CPTcAMP significantly stimulated lumen-to-bath ^{22}Na efflux from 31.0±2.0 to 36.8±1.2 ($n = 8$, $P < 0.025$, ANOVA). No significant difference in ouabain-resistant, passive ^{22}Na permeability between the two groups was observed (2.4 mM Ca^{++} 8.9±2.3 vs 100 nM Ca^{++} 12.8±2.2 pmol · mm^{-1} · min^{-1}). Thus while lowering bath calcium did not affect basal J_{Na}, it converted the inhibitory effect of 8CPTcAMP on J_{Na}(l-b) to a significant stimulatory effect.

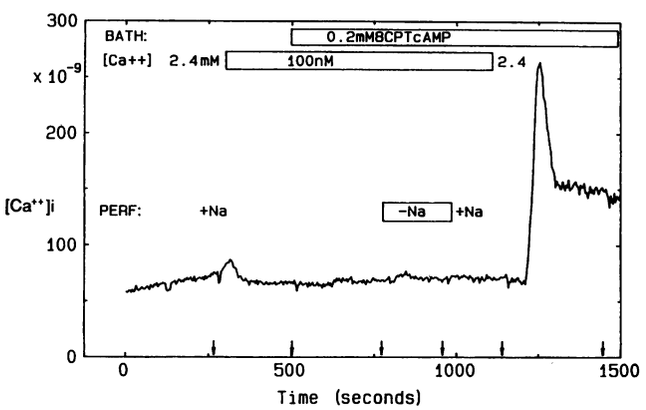

Figure 6. Lowering basolateral $[Ca^{++}]$ blocks the 8CPTcAMP stimulated $[Ca^{++}]_i$ increase. $[Ca^{++}]_i$ is plotted on the left vertical axis in nanomolars. Bath Ca^{++} was lowered from the control concentration of 2.4 mM to 100 nM. This maneuver blocked the increase in $[Ca^{++}]_i$ seen either with exposure to 0.2 mM 8CPTcAMP or the subsequent removal and readdition of luminal Na^+. However when bath Ca^{++} was restored to 2.4 mM in the cAMP-treated CCD, there is a marked increase in $[Ca^{++}]_i$ presumable due to Ca^{++} influx across the basolateral cell membrane.

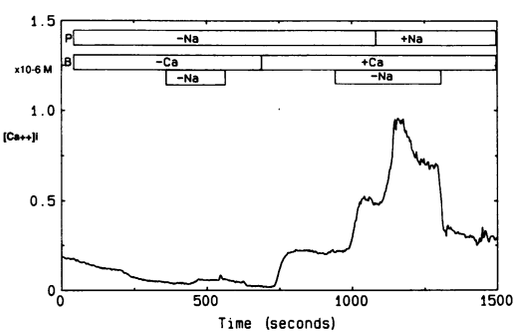

Figure 7. Basolateral sodium removal raises CCD $[Ca^{++}]_i$. $[Ca^{++}]_i$ is plotted on the left vertical axis in micromolar. Sodium was initially absent from the lumen. Bath Ca^{++} was removed, followed by bath sodium removal. No significant change in $[Ca^{++}]_i$ was observed. Bath sodium and Ca^{++} were then sequentially restored. Subsequent removal of bath sodium, now in the presence of bath Ca^{++} causes $[Ca^{++}]_i$ to increase significantly. Readdition of luminal sodium causes a further increase in $[Ca^{++}]_i$. Restoration of bath sodium restores $[Ca^{++}]_i$ to basal levels.

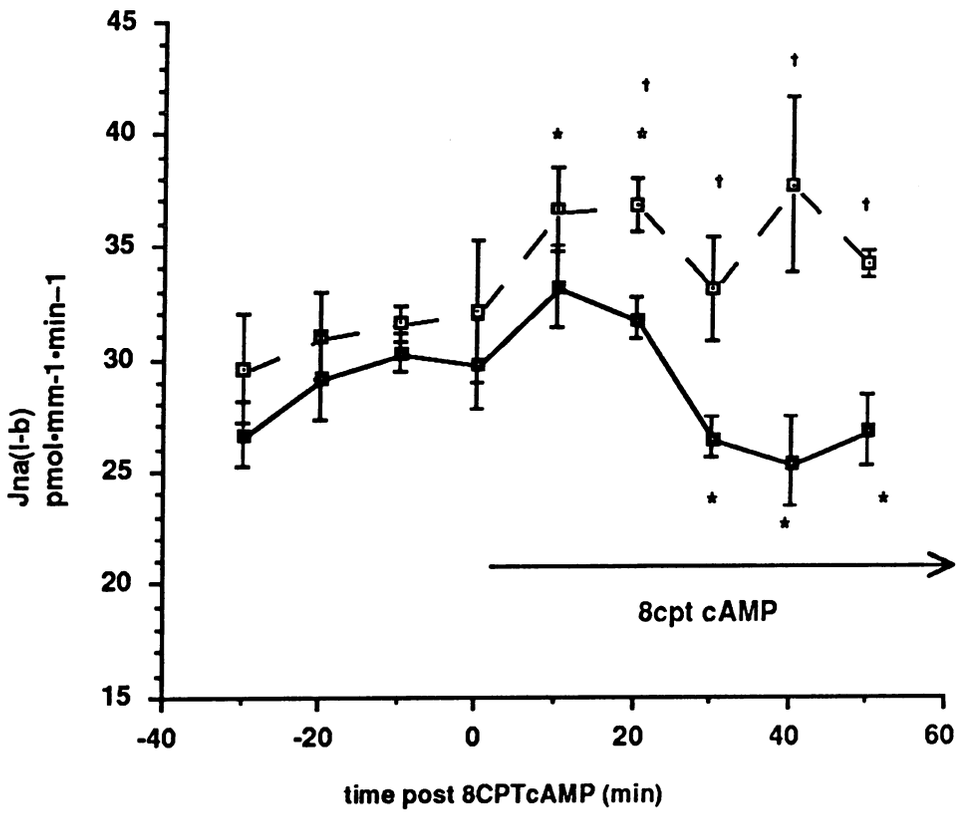

Figure 8. The effect of 0.1 mM 8CPTcAMP on Na^+ transport in either a 2.4 mM (■) or 100 nM (□) Ca^{++} bath. The horizontal axis plots time in minutes before or after the addition of 0.1 mM 8CPTcAMP to the bath. The vertical axis plots J_{Na}(l-b) in $pmol \cdot mm^{-1} \cdot min^{-1}$. The solid line shows the time course of the effect of 8CPTcAMP on J_{Na} in a 2.4 mM Ca bath (n = 7). The dashed line is the same experiment performed in a 100 nM Ca^{++} bath (n = 8). *Significant differences from basal levels within a group. †Significant differences between groups. All comparisons were made by ANOVA, significance $P < 0.05$).

Discussion

The cellular mechanism by which AVP modulates salt and water transport in the collecting duct has been primarily attributed to activation of a V_2 receptor coupled to cAMP generation (3, 6–8, 21, 29, 30). However, recent studies (including the present data) have shown AVP can also increase collecting duct $[Ca^{++}]_i$. This latter effect may result from phosphatidylinositol hydrolysis (14, 15, 31–33). Nanomolar AVP releases Ca^{++} from intracellular stores, an effect consistent with IP_3 generation. The physiologic significance of the AVP-stimulated $[Ca^{++}]_i$ increase or AVP-stimulated phosphatidylinositol hydrolysis is unclear since high concentrations are required and cAMP can mimick all of the functional effects of AVP in the CCD. Importantly, the present studies show that picomolar AVP or cAMP, AVP's classical second messenger, can also increase CCD $[Ca^{++}]_i$. This $[Ca^{++}]_i$ increase is totally dependent on extracellular Ca^{++}, suggesting an IP_3-independent mechanism (27, 33). Thus two signaling pathways are potentially involved in the AVP-stimulated calcium increase:

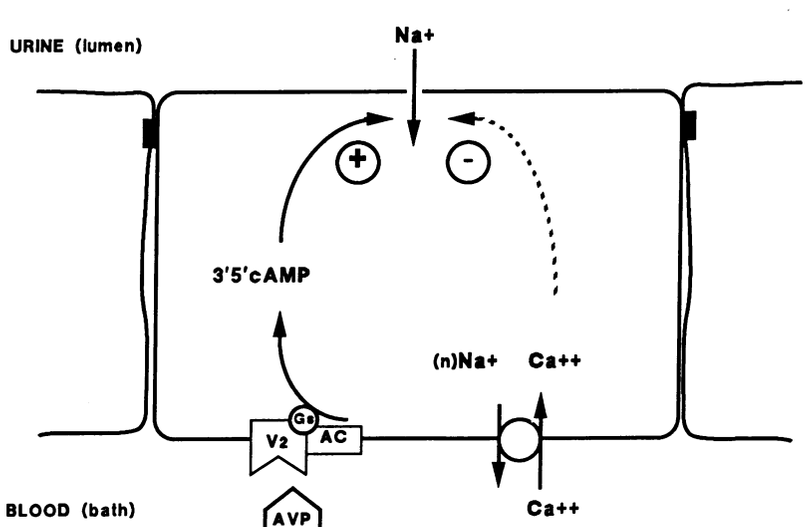

Figure 9. Model for the interactions between AVP, cAMP, luminal Na^+, and basolateral Ca^{++} in the rabbit CCD. Activation of apical CCD cell Na permeability by cAMP leads to increased intracellular Na^+. This alters the electrochemical gradient across a basolateral Na^+/Ca^{++} exchanger leading to increased Ca^{++} influx across the basolateral membrane. Direct or indirect effects of increased $[Ca^{++}]_i$ may then inhibit apical sodium permeability.

cAMP-stimulated calcium entry and a separate pathway coupled to intracellular Ca^{++} release and only activated by high (nanomolar) AVP concentrations.

We next characterized the mechanism of the cAMP stimulated $[Ca^{++}]_i$ increase. Luminal Na^+ removal completely blocked the cAMP-induced $[Ca^{++}]_i$ increase. This suggests that the cAMP-stimulated $[Ca^{++}]_i$ increase may be dependent on a primary change in luminal Na^+ permeability. One mechanism by which luminal Na^+ entry might change $[Ca^{++}]_i$ is via coupling to a putative basolateral Na^+/Ca^{++} exchanger (34) as depicted in Fig. 9. This model would require both that cAMP increase luminal Na^+ permeability and that a basolateral Na^+/Ca^{++} exchanger exist in the rabbit CCD. Two observations in the present studies suggest that cAMP increases luminal Na^+ permeability in the rabbit CCD. These are that (*a*) cAMP increases transepithelial ^{22}Na flux in a 100 nM Ca^{++} bath and (*b*) cAMP augments the $[Ca^{++}]_i$ increase seen after luminal Na^+ readdition by > 20 times the increase in $[Ca^{++}]_i$ seen before cAMP. These observations constitute the first evidence that cAMP can stimulate luminal Na^+ permeability in the rabbit CCD. Several studies suggest that cAMP increases luminal Na^+ permeability in other AVP responsive epithelia (8, 26, 35). Flux studies in toad bladder and electrophysiologic studies in the rat CCD suggest that cAMP increases Na^+ transport by increasing apical cell membrane Na^+ permeability via the amiloride-sensitive Na^+ channel (8, 26, 30, 35).[3] The present studies now link luminal Na^+ entry to an effect of cAMP's on $[Ca^{++}]_i$ in the rabbit CCD.

We also demonstrate that the cAMP $[Ca^{++}]_i$ increase is strictly dependent on basolateral Ca^{++}. The dual dependence of the cAMP $[Ca^{++}]_i$ increase on luminal Na^+ and bath Ca^{++} is consistent with an effect of cAMP on basolateral Na^+/Ca^{++} exchange. More direct evidence for the existance of basolateral Na^+/Ca^{++} exchange comes from studies, performed in the absence of cAMP, showing that peritubular Na^+ removal increases CCD $[Ca^{++}]_i$. The extent to which peritubular Na^+ removal increases $[Ca^{++}]_i$ depends on both peritubular Ca^{++} and luminal Na^+ concentration, characteristics identical to the cAMP-induced $[Ca^{++}]_i$ increase. The $[Ca^{++}]_i$ increase due to peritubular Na^+ removal is completely dependent on extracellular Ca^{++}, documenting that Na^+ removal does not release Ca^{++} from intracellular stores (Fig. 7). These results suggest a Na^+/Ca^{++} exchanger localized on the basolateral membrane. Luminal Na^+/Ca^{++} exchange cannot explain the present results since luminal Na^+ removal does not change $[Ca^{++}]_i$. The results are also inconsistent with cAMP activation of an occult luminal Na^+/Ca^{++} exchanger, since after cAMP, luminal Na^+ readdition increases $[Ca^{++}]_i$ instead of the expected decrease, which would be seen if a luminal Na^+/Ca^{++} exchanger were present. The sodium readdition effect is also dependent on basolateral Ca^{++} (Fig. 6). These results provide evidence for basolateral Na^+/Ca^{++} exchange in the CCD and suggest that cAMP modulates luminal Na^+ entry or its coupling to this exchanger.

Alternate mechanisms for the cAMP $[Ca^{++}]_i$ increase, such as an effect on Ca^{++} channels (27), cannot readily explain the present findings. Channel-mediated $[Ca^{++}]_i$ entry is sensitive to changes in intracellular voltage. Luminal Na^+ readdition should depolarize the negative intracellular voltage (26). This would decrease the electrical driving force for conductive Ca^{++} entry, thereby lowering $[Ca^{++}]_i$. Instead, luminal Na^+ readdition increased $[Ca^{++}]_i$ (Fig. 5). Intracellular depolarization could activate Ca^{++} influx, through voltage-sensitive Ca^{++} channels (27); however, depolarization of the rabbit CCD with a 20 mM K^+ bath also failed to increase $[Ca^{++}]_i$. So the lack of depolarization-induced calcium influx argues against voltage-activated Ca^{++} channels in the rabbit CCD. The existance of Ca^{++} channels in the CCD remains conjectural, but their transport characteristics are incompatible with the present findings.

In contrast, operation of a Na^+/Ca^{++} exchanger as a Ca^{++} influx pathway is well documented, and can account for the present observations (36, 37). Snowdowne et al. (36) have shown the Na^+/Ca^{++} exchanger is a major Ca^{++} influx pathway in cultured kidney LLC-MK_2 cells. In the rabbit CCD we calculated the free energy for Na^+ and Ca^{++} entry across a 3Na/1Ca^{++} exchanger (assuming intracellular Na^+ is 15 mM, basolateral membrane voltage is −84 mV (38) and $[Ca^{++}]_i$ is 100 nM). This yields a free energy for Ca^{++} entry of −42.2 kJ/mol and for 3(Na^+) entry of −42.1 kJ/mol. Under these conditions the electrochemical driving forces across such a Na^+/Ca^{++} exchanger are exactly balanced. Small increments in intracellular Na^+ concentration would lead to Ca^{++} influx. While it remains to be determined whether the cAMP-induced $[Ca^{++}]_i$ increase is due to decreased Ca^{++} efflux or increased influx, it can be predicted that increased cell Na^+ would alter transport across the basolateral Na^+/Ca^{++} exchanger so as to increase $[Ca^{++}]_i$.

An important role for the cAMP-stimulated $[Ca^{++}]_i$ increase in regulating transport is suggested by the differential effect of cAMP on CCD Na^+ transport in a high versus low $[Ca^{++}]_i$ bath. cAMP both increases $[Ca^{++}]_i$ and inhibits Na^+ transport in a 2.4 mM Ca^{++} bath, whereas cAMP does not raise $[Ca^{++}]_i$ and stimulates Na^+ transport in a 100 nM Ca^{++} bath. These observations are consistent with the studies of Frindt and Windhager (11), which show that maneuvers designed to increase CCD $[Ca^{++}]_i$ (quinidine or ionomycin), inhibit luminal Na^+ permeability. So, while cAMP may directly increase Na^+ permeability, the secondary $[Ca^{++}]_i$ increase would feed back to inhibit luminal cell membrane Na^+ entry (Fig. 9). This could explain the close temporal relationship between increased CCD $[Ca^{++}]_i$ and the transient initial negative deflection in V_t produced by AVP or cAMP in the rabbit CCD (Fig. 4). This is also consistent with the brief period where cAMP increases J_{Na}, albeit not significantly ($P < 0.1$ ANOVA Fig. 8), before its inhibitory effect on J_{Na} in 2.4 mM bath Ca^{++}. While the biphasic effect of cAMP on V_t and possibly J_{Na} in normal bath Ca^{++} require further study, it is clear that 8CPTcAMP stimulates J_{Na}(l-b) in a low Ca^{++} bath. The present observations provide the first evidence for hormonal activation of Ca^{++}-dependent feedback control of Na^+ transport in the collecting duct (34, 39).

The observed $[Ca^{++}]_i$ increase may be only indirectly responsible for inhibition of Na^+ absorption in the rabbit CCD. Patch clamp data argues against a direct inhibitory effect of $[Ca^{++}]_i$ on conductance through the amiloride sensitive Na^+ channel (40, 41). Some studies suggest that Ca^{++} dependent activation of other signaling pathways such as cyclooxygenase

3. Luminal amiloride could not be used to independently test for blockade of either 8CPTcAMP effects on the epithelial sodium channel or basolateral Ca^{++} exchange since it is intensely fluorescent at 340 nM excitation.

metabolites or protein kinase C activation, may be involved (28, 40, 41). These possibilities await further testing.

Like AVP, the effects of FSK and 8CPTcAMP on CCD $[Ca^{++}]_i$ appear to be predominantly on the principal cell rather than the intercalated cell. AVP selectively binds to and increases cAMP and $[Ca^{++}]_i$ in the CCD principal cell but not the intercalated cell (15, 23, 42). Conversely, the β-adrenergic agonist isoproterenol selectively stimulates cAMP generation in the intercalated cell but not in the principal cell (23). Since 10^{-4} M isoproterenol failed to increase CCD $[Ca^{++}]_i$, and this concentration maximally stimulates intercalated cell mediated cAMP generation and HCO_3^- secretion (23, 43), it seems unlikely that FSK or 8CPTcAMP raise CCD $[Ca^{++}]_i$ by an effect on the intercalated cell. Furthermore, since the principal cell is thought to be the major site of transepithelial Na^+ permeability in the CCD (26, 44), the finding that luminal Na^+ removal completely blocks the effect of cAMP to increase $[Ca^{++}]_i$, is consistent with an effect of 8CPTcAMP on principal cell $[Ca^{++}]_i$.

Cyclic AMP increases $[Ca^{++}]_i$ in several cells including pituitary cells, toad bladder epithelial cells and striated muscle (45–47). The cellular mechanism is well established only for skeletal muscle, where cAMP-dependent protein kinase phosphorylates a dihydropyridine-sensitive voltage-dependent Ca^{++} channel (39). cAMP has also been shown to increase intracellular Ca^{++} in the rabbit connecting tubule (48). Differences between the cAMP effect on connecting tubule $[Ca^{++}]_i$ and that observed in the rabbit CCD include the fact that the connecting tubule is parathyroid hormone but not AVP sensitive, and that the cAMP effects may be linked to increased active Ca^{++} absorption in the connecting tubule, whereas there is no detectable cAMP-stimulated Ca^{++} transport in the rabbit CCD (49). The present studies are the first in any tissue to suggest that cAMP raises $[Ca^{++}]_i$ secondary to coupling between increased Na^+ influx and Na^+/Ca^{++} exchange.

In summary the present studies, show that: (*a*) 8CPTcAMP inhibits CCD J_{Na}(l-b) in millimolar bath Ca^{++} but stimulates J_{Na}(l-b) when bath Ca^{++} concentration is lowered to 100 nM; (*b*) cAMP analogues, picomolar AVP, dDAVP, or 0.1 mM FSK all increase $[Ca^{++}]_i$ in the rabbit CCD; (*c*) this $[Ca^{++}]_i$ increase is blocked when bath Ca^{++} is 100 nM or less; (*d*) the cAMP stimulated Ca^{++} increase occurs only in the presence of luminal Na^+ and cAMP augments the $[Ca^{++}]_i$ increase seen with luminal Na^+ readdition; (*e*) peritubular, but not luminal, Na^+ removal raises CCD $[Ca^{++}]_i$ but only if basolateral Ca^{++} is present, suggesting the existence of basolateral Na^+/Ca^{++} exchange. We propose that cAMP initially increases apical Na^+ entry, and secondarily raises CCD $[Ca^{++}]_i$ by altering calcium transport across a basolateral Na^+/Ca^{++} exchanger. The primary increase in CCD Na^+ transport produced by cAMP is offset by the secondary increase in $[Ca^{++}]_i$ which contributes to the inhibition of Na^+ absorption produced by vasopressin and cAMP in the rabbit CCD.

Acknowledgments

The author thanks Dr. Harry Jacobson for his scientific advice and continued support. The author also thanks Dr. Gustavo Frindt for his helpful comments and Mr. Shea Tuberty for his technical support.

This work was funded by a Department of Veterans Affairs Research Associate-Career Development and Merit Review award, and NIH-NIDDK award R01 DK37097-06.

References

1. Bartter, F. C., and W. B. Schwartz. 1967. The syndrome of inappropriate secretion of antidiuretic hormone. *Am. J. Med.* 42:790–806.

2. Gross, P. A., and R. J. Anderson. 1982. Effects of dDAVP and AVP on sodium and water balance in conscious rat. *Am. J. Physiol.* 243:R512–519.

3. Grantham, J., and M. B. Burg. 1966. Effect of vasopressin and cyclic AMP on permeability of isolated collecting tubules. *Am. J. Physiol.* 211:255–259.

4. Frindt, G., and M. B. Burg. 1972. Effect of vasopressin on sodium transport in renal cortical collecting tubules. *Kidney Int.* 1:224–231.

5. Holt, W. F., and C. LeChene. 1981. ADH-PGE2 interactions in cortical collecting tubule. I. Depression of sodium transport. *Am. J. Physiol.* 241:F452–460.

6. Orloff, J., and J. S. Handler. 1962. The similarity of effects of vasopressin, adenosine-3'5'-phosphate (cyclic AMP) and theophylline on the toad bladder. *J. Clin. Invest.* 41:702–709.

7. Abramov, M., R. Beauwens, and E. Cogan. 1987. Cellular events in vasopressin action. *Kidney Int.* 32:s56–66.

8. Schafer, J. A., and S. L. Troutman. 1990. cAMP mediates the increase in apical membrane Na^+ conductance produced in rat CCD by vasopressin. *Am. J. Physiol.* 259:F823–831.

9. Jones, S. M., G. Frindt, and E. E. Windhager. 1988. Effect of peritubular [Ca] or ionomycin on hydrosomtic response of CCTs to ADH or cAMP. *Am. J. Physiol.* 254:F240–253.

10. Ando, Y., H. R. Jacobson, and M. D. Breyer. 1988. Phoborl ester and A23187 have additive but mechanistically separate effects on vasopressin action in the rabbit collecting tubule. *J. Clin. Invest.* 81:1578–1584.

11. Frindt, G., and E. E. Windhager. 1990. Ca^{2+}-dependent inhibition of sodium transport in rabbit cortical collecting tubules. *Am. J. Physiol.* 258:F568–582.

12. Star, R. A., H. Nonoguchi, R. Balaban, and M. A. Knepper. 1988. Calcium and cyclic adenosine monophosphate as second messengers for vasopressin in the rat inner medullary collecting duct. *J. Clin. Invest.* 81:1879–1888.

13. Ando, Y., M. D. Breyer, and H. R. Jacobson. 1989. Dose-dependent heterogenous actions of vasopressin in rabbit cortical collecting ducts. *Am. J. Physiol.* 256:F556–562.

14. Teitelbaum, I. 1991. Vasopressin-stimulated phosphoinositide hydrolysis in cultured rat inner medullary collecting duct cells is mediated by the oxytocin receptor *J. Clin. Invest.* 87:2122–2126.

15. Burnatowska-Hledin, M. A., and W. S. Speilman. 1989. Vasopressin V1 receptors on the principal cells of the rabbit cortical collecting tubule. *J. Clin. Invest.* 83:84–89.

16. Kimmel, P. L., and S. Goldfarb. 1984. Effects of isoproterenol on potassium secretion by the cortical collecting tubule. *Am. J. Physiol.* 246:F804–810.

17. Grynkiewicz, G., M. Poenie, and R. Y. Tsien. 1985. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem.* 260:3440–3450.

18. Urbano, E., H. Offenbacher, and O. S. Wolfbeis. 1984. Optical sensor for continuous determination of halides. *Anal. Chem.* 56:427–429.

19. Schuster, V. L. 1985. Mechanism of bradykinin, ADH, and cAMP interaction in rabbit cortical collecting duct. *Am. J. Physiol.* 249:F645–653.

20. Perrin, D. D., and I. G. Sayce. 1967. Computer calculation of equilibrium concentrations in mixtures of metal ions and complexing species. *Talanta.* 14:833–842.

21. Butlen, D., G. Guillon, R. M. Rajerison, S. Jard, W. H. Sawyer, and M. Manning. 1978. Structural requirements for activation of vasopressin-sensitive adenylate cyclase, hormone binding, and antidiuretic actions: effects of highly potent analogues and competitive inhibitors. *Mol. Pharmacol.* 14:1006–1017.

22. Nadler, S. P., S. C. Hebert, and B. M. Brenner. 1986. PGE_2, forskolin, and cholera toxin interactions in rabbit cortical collecting tubule. *Am. J. Physiol.* 250:F127–136.

23. Fejes-Toth, G., and A. Naray-Fejes-Toth. 1989. Isolated principal and intercalated cells: hormone responsiveness and Na^+-K^+-ATPase activity. *Am. J. Physiol.* 256:F742–750.

24. Chabardes, D., M. Imbert-Teboul, M. Montegut, A. Clique, and F. Morel. 1975. Catecholamine sensitive adenylate cyclase activity in different segments of the rabbit nephron. *Pflügers Arch.* 361:9–15.

25. Stoner, L. C., M. B. Burg, and J. Orloff. 1974. Ion transport in cortical collecting tubule: effect of amiloride. *Am. J. Physiol.* 227:453–459.

26. Schlatter, E., and J. A. Schafer. 1987. Electrophysiologic studies in principal cells of rat cortical collecting tubules: ADH increases the apical membrane Na^+ conductance. *Pflügers Arch.* 409:81–92.

27. Carafoli, E. 1987. Intracellular calcium homeostasis. *Annu. Rev. Biochem.* 56:395–433.

28. Hebert, R. L., H. R. Jacobson, and M. D. Breyer. 1991. Prostaglandin E_2 inhibits sodium transport in the rabbit CCD by raising intracellular calcium. *J. Clin. Invest.* 87:1992–1998.

29. Guillon, G., D. Butlen, B. Antau, T. Barth, and S. Jard. 1982. Kinetic and

pharmacological characterization of vasopressin membrane receptors from human kidney medulla: relation to adenylate cyclase activation. *Eur. J. Pharmacol.* 85:291–304.

30. Reif, M. C., S. L. Troutman, and J. A. Schafer. 1986. Sodium transport by rat cortical collecting tubule: effects of vasopressin and desoxycorticosterone. *J. Clin. Invest.* 77:1291–1298.

31. Michell, R. H., C. J. Kirk, and M. M. Billah. 1979. Hormonal stimulation of phosphatidylinositol breakdown with particular reference to the hepatic effects of vasopressin. *Biochem. Soc. Trans.* 7:861–865.

32. Aiyar, N., P. Nambi, F. L. Stassen, and S. T. Crooke. 1986. Vascular vasopressin receptors mediate phosphatidylinositol turnover and calcium efflux in an established smooth muscle cell line. *Life Sci.* 39:37–45.

33. Berridge, M. J. 1987. Inositol trisphosphate and diacylglycerol: two interacting second messengers. *Annu. Rev. Biochem.* 56:159–193.

34. Taylor, A., and E. E. Windhager. 1985. Cytosolic calcium and its role in the regulation of transepithelial ion and water transport. *In* Physiology and Pathophysiology of Electrolyte Metabolism. D. Seldin and G. Giebisch, editors. Raven Press, New York. 1297–1397.

35. Garty, H., and D. J. Benos. 1988. Characteristics and regulatory mechanisms of the amiloride-blockable Na^+ channel. *Physiol. Rev.* 68:309–373.

36. Snowdowne, K. W., and A. B. Borle. 1985. Effect of low extracellular sodium on cytosolic ionized calcium: Na^+-Ca^+ exchange as a major calcium influx pathway in kidney cells. *J. Biol. Chem.* 260:14998–15007.

37. Bourdeau, J. E., and K. Lau. 1990. Basolateral cell membrane Ca-Na exchange in single rabbit connecting tubules. *Am. J. Physiol.* 258:F1497–1503.

38. Koeppen, B. M., B. A. Biagi, and G. H. Giebisch. 1983. Intracellular microelectrode characterization of the rabbit cortical collecting duct. *Am. J. Physiol.* 244:F35–47.

39. Chase, H. S. 1984. Does calcium couple the apical and basolateral membrane permeabilities in epithelia? *Am. J. Physiol.* 247:F869–876.

40. Ling, B., and D. C. Eaton. 1989. Effects of luminal Na^+ on single Na^+ channels in A6 cells, a regulatory role for protein kinase C. *Am. J. Physiol.* 256:F1094–F1103.

41. Palmer, L. G., and G. Frindt. 1987. Effects of cell Ca and pH on Na channels from rat cortical collecting tubule. *Am. J. Physiol.* 253:F333–339.

42. Kirk, K. L., A. Buku, and P. Eggena. 1987. Cell specificity of vasopressin binding in renal collecting duct: computer-enhanced imaging of a fluorescent hormone analog. *Proc. Natl. Acad. Sci. USA.* 84:6000–6004.

43. Schuster, V. L. 1985. Cyclic Adenosine monophosphate-stimulated bicarbonate secretion in rabbit cortical collecting tubules. *J. Clin. Invest.* 75:2056–2064.

44. O'Neil, R. G., and R. H. Hayhurst. 1985. Functional differentiation of cell types of the cortical collecting duct. *Am. J. Physiol.* 248:F449–453.

45. Catteral, W. A., M. J. Seagar, and M. Takahashi. 1988. Molecular properties of dihydropyridine-sensitive calcium channels in skeletal muscle. *J. Biol. Chem.* 263:3535–3538.

46. Reisine, T., and S. Guild. 1987. Activators of protein kinase C and cyclic AMP-dependent protein kinase regulate intracellular calcium through distinct mechanisms in mouse anterior pituitary tumor cell. *Mol. Pharmacol.* 32:488–496.

47. Wong, M. E., and H. S. Chase. 1988. Effect of vasopressin on intracellular [Ca] and Na transport in cultured toad bladder cells. *Am. J. Physiol.* 255:F1015–1024.

48. Lau, K., and J. E. Bourdeau. 1989. Evidence for cAMP-dependent protein kinase in mediating the parathyroid hormone-stimulated rise in cytosolic free calcium in rabbit connecting tubules. *J. Biol. Chem.* 264:4028–4032.

49. Imai, M. 1981. Effects of parathyroid hormone and N^6,O^2-dibutyryl cyclic AMP on Ca^{2+} transport across the rabbit distal nephron segments perfused in vitro. *Pflügers Arch.* 390:145–151.