

## Feedback inhibition of cyclic adenosine monophosphate-stimulated Na<sup>+</sup> transport in the rabbit cortical collecting duct via Na(+)-dependent basolateral Ca<sup>++</sup> entry.

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### Research Article

Arginine vasopressin (AVP) transiently stimulates Na<sup>+</sup> 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<sup>+</sup> transport in the rabbit CCD is inhibitory. Because maneuvers that increase [Ca<sup>++</sup>]<sub>i</sub> inhibit Na<sup>+</sup> transport, the effects of AVP and cell-permeable cAMP analogues, on [Ca<sup>++</sup>]<sub>i</sub> were investigated in fura-2-loaded in vitro microperfused rabbit CCDs. Low-dose AVP (23-230 pM) selectively stimulated Ca<sup>++</sup> 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<sup>++</sup>]<sub>i</sub>. The 8CPTcAMP-stimulated [Ca<sup>++</sup>]<sub>i</sub> increase was totally dependent on basolateral [Ca<sup>++</sup>]. In the absence of cAMP, peritubular Na<sup>+</sup> removal produced a marked increase in [Ca<sup>++</sup>]<sub>i</sub>, which was also dependent on bath [Ca<sup>++</sup>], suggesting the existence of basolateral Na<sup>+</sup>/Ca<sup>++</sup> exchange. Luminal Na<sup>+</sup> removal in the absence of cAMP did not alter CCD [Ca<sup>++</sup>]<sub>i</sub>, but it completely blocked the cAMP-stimulated [Ca<sup>++</sup>]<sub>i</sub> increase. Thus the cAMP-dependent Ca<sup>++</sup> increase is totally dependent on both luminal Na<sup>+</sup> and basolateral Ca<sup>++</sup>, suggesting the [Ca<sup>++</sup>]<sub>i</sub> increase is secondary to cAMP effects on luminal Na<sup>+</sup> entry and its coupling to basolateral Na<sup>+</sup>/Ca<sup>++</sup> exchange. 8CPTcAMP inhibits lumen-to-bath 22Na flux [<sup>22</sup>Na(I-b)] in CCDs bathed in a normal Ca<sup>++</sup> bath (2.4 mM). However, when bath Ca<sup>++</sup> was lowered to 100 nM, a maneuver that also blocks the 8CPTcAMP [Ca<sup>++</sup>]<sub>i</sub> [...]

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# Feedback Inhibition of Cyclic Adenosine Monophosphate-stimulated Na<sup>+</sup> Transport in the Rabbit Cortical Collecting Duct via Na<sup>+</sup>-dependent Basolateral Ca<sup>++</sup> Entry

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## Abstract

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

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## Introduction

Arginine vasopressin (AVP)<sup>1</sup> 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<sup>++</sup> concentration ([Ca<sup>++</sup>]<sub>i</sub>) plays an important role in AVP's action (9–15). Nanomolar AVP has been shown to increase [Ca<sup>++</sup>]<sub>i</sub> in isolated collecting duct cells (13–15). Burnatowska-Hledin et al. (15) have found 10<sup>-6</sup> M AVP stimulates inositol phosphate formation in immunodissected rabbit cortical collecting duct (CCD) cells and have attributed the Ca<sup>++</sup> 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<sup>++</sup>]<sub>i</sub> increase (14), and suggests a singular role for cAMP signaling in mediating AVP's effects in the CCD. Nevertheless, maneuvers that increase [Ca<sup>++</sup>]<sub>i</sub> 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<sup>++</sup>]<sub>i</sub> 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<sub>3</sub>, 25; Na acetate, 10; Na<sub>2</sub>HPO<sub>4</sub>, 2.3; KCl, 5; CaCl<sub>2</sub>, 2.4; MgSO<sub>4</sub>, 1.0 glucose, 8.3; and alanine, 5 (osmolality 300 mosmol). Sodium-free solutions were made by substituting sodium salts with tetramethylammonium salts. HCO<sub>3</sub><sup>-</sup> was generated by bubbling tetramethylammonium-OH with 5% CO<sub>2</sub>/95% O<sub>2</sub>. Ca<sup>++</sup>-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<sup>++</sup>.

Transepithelial voltage (V<sub>t</sub>) 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<sub>t</sub> (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<sup>++</sup>]<sub>i</sub> 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 monochromators set at 340 and 380 nm, respectively (Deltascan, Photon Technology International, New Brunswick, NJ). The monochromator 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<sup>++</sup>]<sub>i</sub> was performed. The bath medium was changed to a Ca<sup>++</sup>- and Mg<sup>++</sup>-free isotonic solution containing EGTA and 10 μM 4Br-A23187. After a stable 340/380 ratio (minimum ratio, R<sub>min</sub>) was achieved the bath was changed back to the 2.4 mM Ca<sup>++</sup> medium plus 10 μM 4Br-A23187 and the ratio was again allowed to stabilize (maximum ratio, R<sub>max</sub>).

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<sub>Na</sub>) in isolated perfused CCDs

Experiments examining CCD sodium transport (J<sub>Na</sub>) used 25 μCi/ml luminal <sup>22</sup>Na and 100 μCi/ml of [<sup>3</sup>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<sub>Na</sub>. 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<sub>Na</sub> measurements was made after 20 min pretreatment with 10<sup>-4</sup> M peritubular ouabain. Ouabain inhibits net J<sub>Na</sub> close to zero (19), and thus the residual lumen-to-bath (l-b) J<sub>Na</sub> is passive and comparable to bath-to-lumen (b-l) J<sub>Na</sub>.

#### Calculations

**Intracellular Ca<sup>++</sup> concentration.** Intracellular Ca<sup>++</sup> concentration ([Ca<sup>++</sup>]<sub>i</sub>) was calculated by: [Ca<sup>++</sup>]<sub>i</sub> = K<sub>d</sub> (R-R<sub>min</sub>)/(R<sub>max</sub>-R) (380 min/380 max) assuming that the K<sub>d</sub> value for the fura-2-Ca<sup>++</sup> complex is 224 nM at 37°C (17).

**Calculation of calcium activity in Ca<sup>++</sup>/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.

**<sup>22</sup>Na flux.** J<sub>Na</sub>(l-b) was calculated from the rate of disappearance of tracer from the perfusate, using the following equation: J<sub>Na</sub>(l-b) = (1 - VoC\*o/ViC\*i) × 145 × L<sup>-1</sup> = pmol · mm<sup>-1</sup> · min<sup>-1</sup>, where C\*i and C\*o are perfused and collected fluid concentrations of <sup>22</sup>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 <sup>22</sup>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<sup>++</sup> bath groups, data for each tubule were normalized to the mean for that group. The J<sub>Na</sub> for a given collection was multiplied by the basal J<sub>Na</sub> for that tubule divided by the mean basal J<sub>Na</sub> 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. <sup>22</sup>Na and [<sup>3</sup>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<sup>++</sup>]<sub>i</sub>.** 8CPTcAMP (0.1, 0.2, or 1 mM) increased CCD [Ca<sup>++</sup>]<sub>i</sub> (Table I, Fig. 1 A). Within 30 s of addition of 0.2 mM 8CPTcAMP [Ca<sup>++</sup>]<sub>i</sub> began to increase and peak [Ca<sup>++</sup>]<sub>i</sub> 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<sup>++</sup>]<sub>i</sub>

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

Bath $[Ca^{++}]$	2.4 mM		<1 nM	
	Basal	Agonist stimulated (n)	Basal	Agonist stimulated (n)
Agonist	Intracellular $[Ca^{2+}]$ , nM			
AVP				
$2 \times 10^{-11}$ M	90±4	125±10* (9)	—	—
$2 \times 10^{-10}$ M	74±19	136±19* (12)	45±5	62±9 (4)
$2 \times 10^{-8}$ 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				
$1 \times 10^{-4}$ M	78±11	160±3* (3)	—	—
$2 \times 10^{-4}$ M	149±45	293±90* (8)	61±12	72±8 (5)
$1 \times 10^{-3}$ M	89±43.4	200±70* (4)	—	—

\*  $P < 0.05$  vs. basal.

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).<sup>2</sup> 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

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.

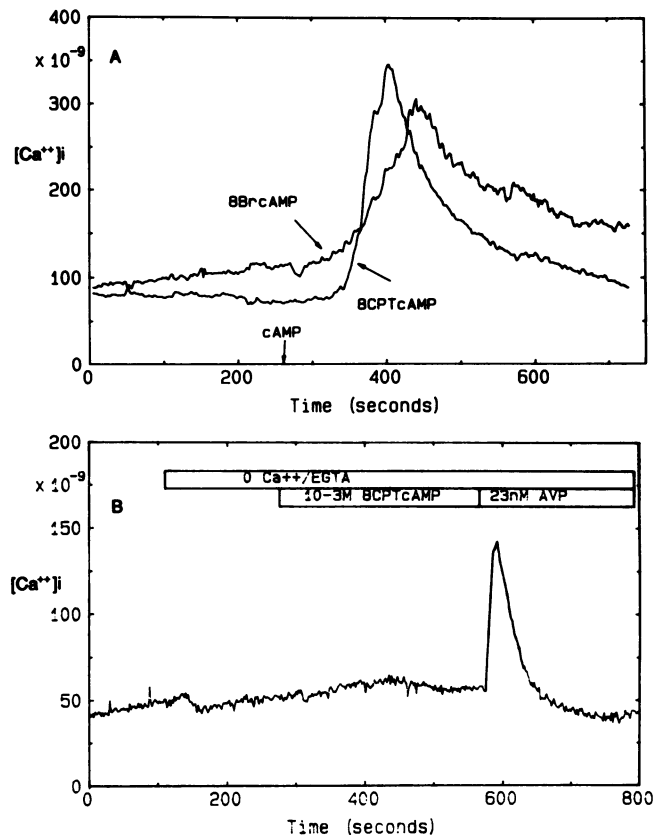


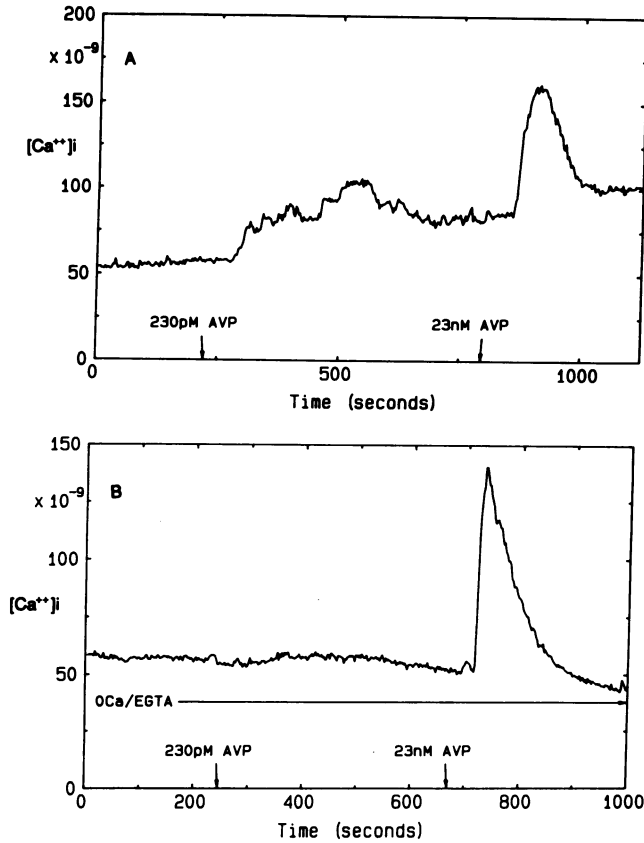
Figure 1. Cell-permeable cAMP analogues increase  $[Ca^{++}]_i$  influx.  $[Ca^{++}]_i$  is plotted on the vertical axis in nanomolars. (A) Effect of either bath 8CPTcAMP (0.2 mM) or 8-Br-cAMP (0.2 mM) on  $[Ca^{++}]_i$ . Analogues were added at the time denoted by the arrow labeled cAMP. (B) 1 mM 8CPTcAMP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

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  $\mu$ M 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,  $P < 0.05$ ,  $n = 6$ ).

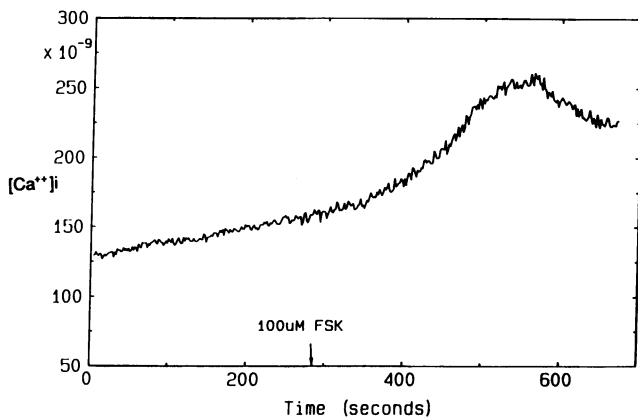
**Effect of isoproterenol on CCD  $[Ca^{++}]_i$ .** Because isoproterenol significantly increases CCD cAMP generation (23, 24), we tested whether isoproterenol also increased  $[Ca^{++}]_i$  in the CCD. In contrast to the effects of AVP, dDAVP, FSK, or cell-permeable cAMP analogues, no significant effect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

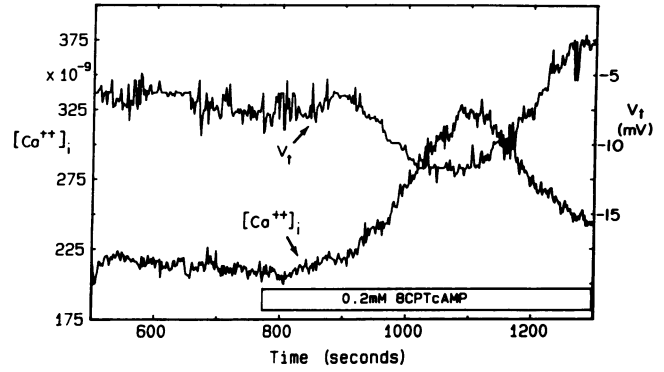


**Figure 2.** Dose-dependent effect of AVP on CCD  $[Ca^{++}]_i$ .  $[Ca^{++}]_i$  is plotted on the left vertical axis in nanomolars. (A) Effects of 230 pM (100  $\mu$ U/ml) and 23 nM (10 mU/ml) AVP in the presence of extracellular  $Ca^{++}$ . (B) Similar experiment conducted in the absence of extracellular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



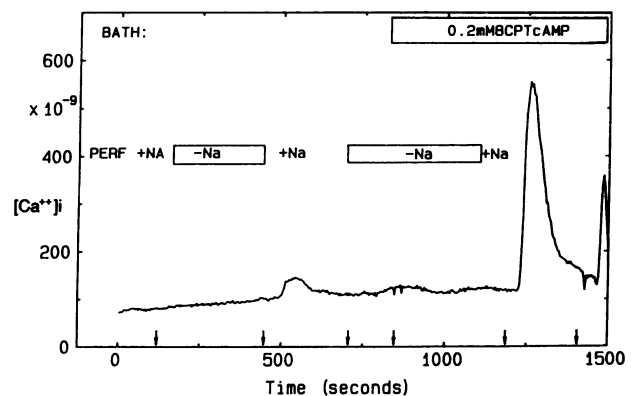
**Figure 3.** FSK increases CCD  $[Ca^{++}]_i$ . The effect of 100  $\mu$ M forskolin on  $[Ca^{++}]_i$  in the CCD. FSK was administered from the bath and at the labeled arrow.  $[Ca^{++}]_i$  is plotted on the left vertical axis in nanomolars.



**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  $155 \pm 55.4$  to  $185 \pm 47.8$ ,  $P = ns$ ). 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  $162 \pm 46.3$  to  $157 \pm 48$ ,  $P = ns$ ). However, when luminal  $Na^+$  was readded to the 8CPTcAMP-treated CCD, there was a 23-fold greater increase in  $[Ca^{++}]_i$  (from  $157 \pm 48$  to  $855 \pm 251$ ,  $P < 0.05$ , 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<sup>+</sup> and Ca<sup>++</sup> removal on [Ca<sup>++</sup>]<sub>i</sub> in Untreated and 8CPTcAMP-treated CCDs

Maneuver	Δ[Ca <sup>++</sup> ] <sub>i</sub> , nM	n
Untreated		
Basolateral Na <sup>+</sup> removal (+ bath Ca <sup>++</sup> )	440±114*	5
Basolateral Na <sup>+</sup> removal (- bath Ca <sup>++</sup> )	67±34	7
Luminal Na <sup>+</sup> removal	9±5	4
Luminal Na <sup>+</sup> readdition	30±15	4
+8-CPT-cAMP		
8CPTcAMP (control)	147±48*	8
8CPTcAMP (- luminal Ca <sup>++</sup> )	55.5±13**§	8
8CPTcAMP (100 nM bath Ca <sup>++</sup> )	5±3‡	5
8CPTcAMP (- luminal Na <sup>+</sup> )	-6±5‡	4
8CPTcAMP luminal Na <sup>+</sup> readdition	698±212*†	4

\* P < 0.05 vs. basal.

‡ P < 0.05 vs. 8CPTcAMP (control).

§ P < 0.05 vs. basolateral Ca<sup>++</sup> removal.

† P < 0.05 vs. Na<sup>+</sup> readdition without 8CPTcAMP.

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

**Polarity of 8CPTcAMP-stimulated Ca<sup>++</sup> influx.** We examined the effect of 0.2–1 mM 8CPTcAMP on [Ca<sup>++</sup>]<sub>i</sub> after either peritubular or luminal Ca<sup>++</sup> was selectively removed (0 Ca<sup>++</sup>/2 mM EGTA buffer) (Table II). In CCDs perfused with Ca<sup>++</sup>-free buffer, 0.2 mM 8CPTcAMP significantly increased [Ca<sup>++</sup>]<sub>i</sub> (basal 135±34.5 to +8CPTcAMP 190.5±39.1, n = 8, P < 0.05). However, when peritubular Ca<sup>++</sup> was selectively removed, 8CPTcAMP failed to increase [Ca<sup>++</sup>]<sub>i</sub> (Table II). Similarly low-

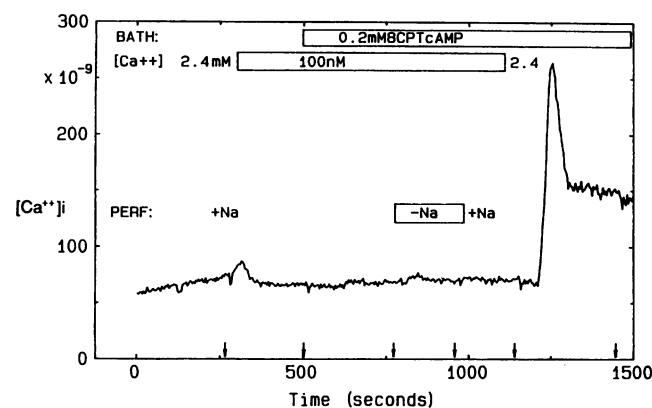


Figure 6. Lowering basolateral [Ca<sup>++</sup>] blocks the 8CPTcAMP stimulated [Ca<sup>++</sup>]<sub>i</sub> increase. [Ca<sup>++</sup>]<sub>i</sub> is plotted on the left vertical axis in nanomolar. Bath Ca<sup>++</sup> was lowered from the control concentration of 2.4 mM to 100 nM. This maneuver blocked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

ering peritubular [Ca<sup>++</sup>] to 100 nM prevented the effect of 8CPTcAMP pretreatment and luminal Na<sup>+</sup> readdition on [Ca<sup>++</sup>]<sub>i</sub> (Fig. 6). Readdition of 2.4 mM bath Ca<sup>++</sup> to 8CPTcAMP-treated CCDs produced a prompt increase in [Ca<sup>++</sup>]<sub>i</sub> above baseline. Thus, 8CPTcAMP significantly increases basolateral Ca<sup>++</sup> influx, but only when Na<sup>+</sup> is present in the lumen.

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

**Role of bath Ca<sup>++</sup> 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<sup>++</sup>] (Fig. 8). In a 2.4 mM Ca<sup>++</sup> bath, 0.1 mM 8CPTcAMP caused a statistically insignificant increase in J<sub>Na</sub>(l-b) over the first 20 min and subsequently J<sub>Na</sub>(l-b) fell from a basal of 30.3±1.0 to 25.4±2 pmol · mm<sup>-1</sup> · min<sup>-1</sup> (n = 7, P < 0.05, ANOVA). In a 100 nM Ca<sup>++</sup> bath, (a condition which blocks the 8CPTcAMP-induced [Ca<sup>++</sup>]<sub>i</sub> increase) 0.1 mM 8CPTcAMP significantly stimulated lumen-to-bath <sup>22</sup>Na eflux from 31.0±2.0 to 36.8±1.2 (n = 8, P < 0.025, ANOVA). No significant difference in ouabain-resistant, passive <sup>22</sup>Na permeability between the two groups was observed (2.4 mM Ca<sup>++</sup> 8.9±2.3 vs 100 nM Ca<sup>++</sup> 12.8±2.2 pmol · mm<sup>-1</sup> · min<sup>-1</sup>). Thus while lowering bath calcium did not affect basal J<sub>Na</sub>, it converted the inhibitory effect of 8CPTcAMP on J<sub>Na</sub>(l-b) to a significant stimulatory effect.

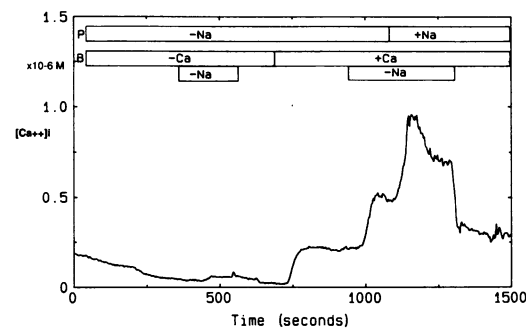


Figure 7. Basolateral sodium removal raises CCD [Ca<sup>++</sup>]<sub>i</sub>. [Ca<sup>++</sup>]<sub>i</sub> is plotted on the left vertical axis in micromolar. Sodium was initially absent from the lumen. Bath Ca<sup>++</sup> was removed, followed by bath sodium removal. No significant change in [Ca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

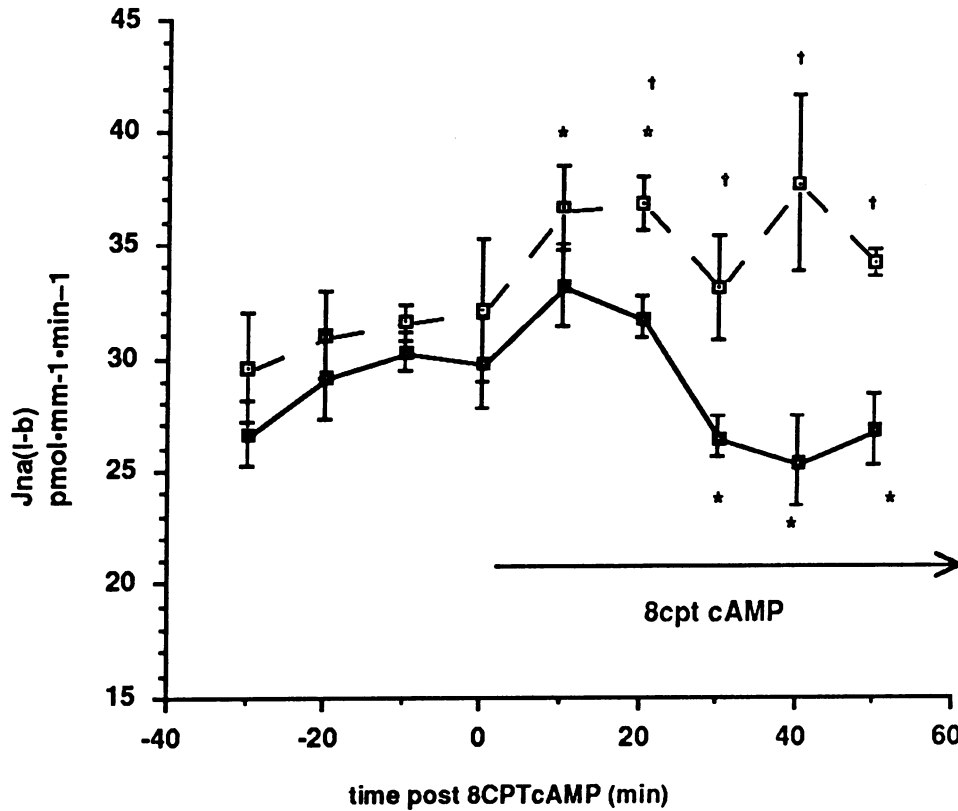


Figure 8. The effect of 0.1 mM 8CPTcAMP on Na<sup>+</sup> transport in either a 2.4 mM (■) or 100 nM (□) Ca<sup>++</sup> bath. The horizontal axis plots time in minutes before or after the addition of 0.1 mM 8CPTcAMP to the bath. The vertical axis plots J<sub>Na</sub>(l-b) in pmol·mm<sup>-1</sup>·min<sup>-1</sup>. The solid line shows the time course of the effect of 8CPTcAMP on J<sub>Na</sub> in a 2.4 mM Ca bath (n = 7). The dashed line is the same experiment performed in a 100 nM Ca<sup>++</sup> 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<sub>2</sub> 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<sup>++</sup>]<sub>i</sub>. This latter effect may result from phosphatidylinositol hydrolysis (14, 15, 31-33). Nanomolar AVP releases Ca<sup>++</sup> from intracellular stores, an effect consistent with IP<sub>3</sub> genera-

tion. The physiologic significance of the AVP-stimulated [Ca<sup>++</sup>]<sub>i</sub> 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<sup>++</sup>]<sub>i</sub>. This [Ca<sup>++</sup>]<sub>i</sub> increase is totally dependent on extracellular Ca<sup>++</sup>, suggesting an IP<sub>3</sub>-independent mechanism (27, 33). Thus two signaling pathways are potentially involved in the AVP-stimulated calcium increase:

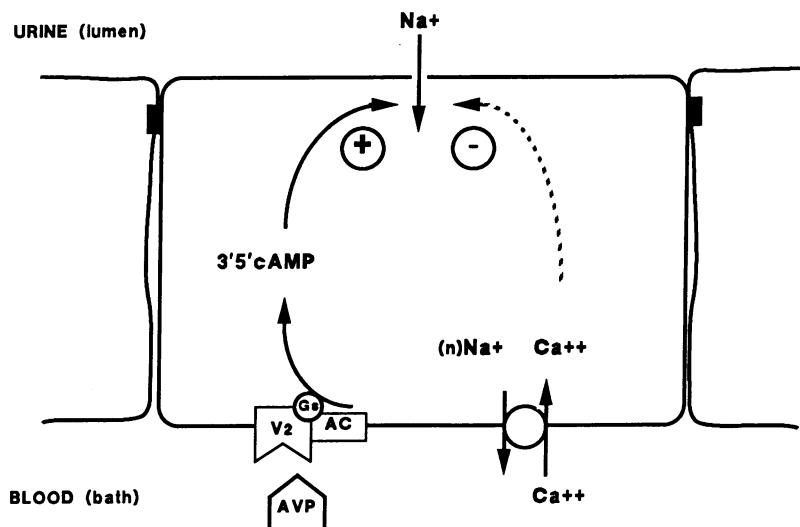


Figure 9. Model for the interactions between AVP, cAMP, luminal Na<sup>+</sup>, and basolateral Ca<sup>++</sup> in the rabbit CCD. Activation of apical CCD cell Na permeability by cAMP leads to increased intracellular Na<sup>+</sup>. This alters the electrochemical gradient across a basolateral Na<sup>+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

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

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

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

Alternate mechanisms for the cAMP  $[\text{Ca}^{++}]_i$  increase, such

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

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

In contrast, operation of a  $\text{Na}^+/\text{Ca}^{++}$  exchanger as a  $\text{Ca}^{++}$  influx pathway is well documented, and can account for the present observations (36, 37). Snowdowne et al. (36) have shown the  $\text{Na}^+/\text{Ca}^{++}$  exchanger is a major  $\text{Ca}^{++}$  influx pathway in cultured kidney LLC-MK<sub>2</sub> cells. In the rabbit CCD we calculated the free energy for  $\text{Na}^+$  and  $\text{Ca}^{++}$  entry across a  $3\text{Na}^+/\text{Ca}^{++}$  exchanger (assuming intracellular  $\text{Na}^+$  is 15 mM, basolateral membrane voltage is  $-84$  mV (38) and  $[\text{Ca}^{++}]_i$  is 100 nM). This yields a free energy for  $\text{Ca}^{++}$  entry of  $-42.2$  kJ/mol and for  $3(\text{Na}^+)$  entry of  $-42.1$  kJ/mol. Under these conditions the electrochemical driving forces across such a  $\text{Na}^+/\text{Ca}^{++}$  exchanger are exactly balanced. Small increments in intracellular  $\text{Na}^+$  concentration would lead to  $\text{Ca}^{++}$  influx. While it remains to be determined whether the cAMP-induced  $[\text{Ca}^{++}]_i$  increase is due to decreased  $\text{Ca}^{++}$  efflux or increased influx, it can be predicted that increased cell  $\text{Na}^+$  would alter transport across the basolateral  $\text{Na}^+/\text{Ca}^{++}$  exchanger so as to increase  $[\text{Ca}^{++}]_i$ .

An important role for the cAMP-stimulated  $[\text{Ca}^{++}]_i$  increase in regulating transport is suggested by the differential effect of cAMP on CCD  $\text{Na}^+$  transport in a high versus low  $[\text{Ca}^{++}]_i$  bath. cAMP both increases  $[\text{Ca}^{++}]_i$  and inhibits  $\text{Na}^+$  transport in a 2.4 mM  $\text{Ca}^{++}$  bath, whereas cAMP does not raise  $[\text{Ca}^{++}]_i$  and stimulates  $\text{Na}^+$  transport in a 100 nM  $\text{Ca}^{++}$  bath. These observations are consistent with the studies of Frindt and Windhager (11), which show that maneuvers designed to increase CCD  $[\text{Ca}^{++}]_i$  (quinidine or ionomycin), inhibit luminal  $\text{Na}^+$  permeability. So, while cAMP may directly increase  $\text{Na}^+$  permeability, the secondary  $[\text{Ca}^{++}]_i$  increase would feed back to inhibit luminal cell membrane  $\text{Na}^+$  entry (Fig. 9). This could explain the close temporal relationship between increased CCD  $[\text{Ca}^{++}]_i$  and the transient initial negative deflection in  $V_i$  produced by AVP or cAMP in the rabbit CCD (Fig. 4). This is also consistent with the brief period where cAMP increases  $J_{\text{Na}}$ , albeit not significantly ( $P < 0.1$  ANOVA Fig. 8), before its inhibitory effect on  $J_{\text{Na}}$  in 2.4 mM bath  $\text{Ca}^{++}$ . While the biphasic effect of cAMP on  $V_i$  and possibly  $J_{\text{Na}}$  in normal bath  $\text{Ca}^{++}$  require further study, it is clear that 8CPTcAMP stimulates  $J_{\text{Na}}$ (1-b) in a low  $\text{Ca}^{++}$  bath. The present observations provide the first evidence for hormonal activation of  $\text{Ca}^{++}$ -dependent feedback control of  $\text{Na}^+$  transport in the collecting duct (34, 39).

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



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  $\beta$ -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.

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