Intracellular Signaling in the Regulation of Renal Na-K-ATPase

I. Role of Cyclic AMP and Phospholipase A_2

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Abstract

We have reported that dopamine (DA) inhibits Na-K-ATPase activity in the cortical collecting duct (CCD) by stimulating the DA_1 receptor, and the present study was designed to evaluate the mechanism of this effect. Short-term exposure (15–30 min) of microdissected rat CCD to DA, a DA_1 agonist (fenoldopam), vasopressin (AVP), forskolin, or dibutyryl cAMP (dBcAMP), which increase cAMP content by different mechanisms, strongly (~ 60%) inhibited Na-K-ATPase activity. 2',5'-dideoxyadenosine, an inhibitor of adenylate cyclase, completely blocked Na-K-ATPase inhibition by DA or fenoldopam, and IP_{20}, an inhibitor peptide of cAMP-dependent protein kinase A (PKA), abolished the Na:K pump effect of all the cAMP agonists listed above. To verify whether the mechanism of pump inhibition by agents that increase cell cAMP involves phospholipase A_2 (PLA_2), we used mepacrine, a PLA_2 inhibitor, which also abolished Na-K-ATPase inhibition by DA or fenoldopam, as well as by AVP, forskolin, or dBcAMP. Arachidonic acid (10^{-7}-10^{-4} M) inhibited Na-K-ATPase activity in dose-dependent fashion. Corticosterone, which induces lipomodulin, a PLA_2 inhibitor protein inactivated by PKA, equally abolished the pump effects of DA, fenoldopam, forskolin, and dBcAMP, suggesting that lipomodulin might act between PKA and PLA_2 in cAMP-dependent pump regulation. We conclude that dopamine inhibits Na-K-ATPase activity in the CCD through a DA_1 receptor-mediated cAMP-PKA pathway that involves the stimulation of PLA_2 and arachidonic acid release, possibly mediated by inactivation of lipomodulin. This pathway is shared by other agonists that increase cell cAMP and thus stimulate PKA activity. (*J. Clin. Invest.* 1992. 89:1496–1500.) Key words: Na:K pump • dopamine • protein kinase A • arachidonic acid • cortical collecting duct

Introduction

Dopamine decreases tubular sodium reabsorption (1, 2), an effect attributed in part to inhibition of Na-K-ATPase activity in the proximal convoluted tubule (PCT)[1] (3, 4). The renal actions of dopamine are mediated by specific receptors (5, 6) classified into the fenoldopam (DA_1) and DA_2 subtypes (reviewed in reference 7). DA_1 receptors, the predominant subtype in renal tubules (7), are located primarily in the PCT (8–10). However, we have recently described specific DA_1 binding also in distal nephron segments, and inhibition of Na-K-ATPase activity in the cortical collecting duct (CCD), where the final regulation of sodium excretion is carried out (11).

Dopamine stimulates adenylate cyclase (8, 10) or cAMP accumulation (4) via its DA_1 receptor. cAMP has been shown to inhibit Na-K-ATPase activity in the liver (12), brain (13), and pancreatic islets (14), but the role of DA_1 receptor activation or of cAMP in the regulation of renal Na-K-ATPase is not clear. In PCT, simultaneous activation of the DA_1 and DA_2 receptor is required, and dibutyryl cAMP (dBcAMP) alone does not inhibit the pump activity (4). In contrast, the DA_1 receptor and cAMP-dependent protein kinase (PKA) have been implicated in pump inhibition in medullary thick ascending limbs (MTAL) (15), where dBcAMP inhibited Na-K-ATPase activity (16), although there are no reports concerning the effect of DA on cAMP accumulation in this nephron segment. We have recently demonstrated parallel actions of DA (mediated via its DA_1 receptor) on cAMP accumulation and on the Na:K pump in CCD (Takemoto, F., H. T. Cohen, T. Satoh, and A. I. Katz, manuscript submitted for publication), raising the possibility that the pump inhibition may be mediated by the cAMP-dependent pathway. That such a mechanism of Na-K-ATPase regulation might involve activation of phospholipase A_2 (PLA_2) and the consequent increase in arachidonic acid release is suggested by observations that cytochrome P450-related arachidonate metabolites generated by a cAMP-dependent mechanism inhibit Na-K-ATPase (17), and that cAMP inhibits sodium transport in CCD through a cyclooxygenase-dependent mechanism (18). The present study was therefore designed to evaluate the mechanism of Na-K-ATPase inhibition by DA and other agonists that increase cell cAMP in microdissected rat CCD by assessing the role of the cAMP-PKA pathway and of PLA_2 in this process.

Methods

Microdissection. Kidneys were obtained from male Sprague-Dawley rats weighing 200–300 g that had free access to regular laboratory chow and tap water. The procedure for tubule microdissection has been reported previously in detail (19). After anesthesia, the left kidney was perfused in situ through a catheter placed in the left renal artery with cold collagenase solution, a modified HBSS containing (mM) 137 NaCl, 5 KCl, 0.8 $MgCl_2$, 0.33 Na_2HPO_4, 0.44 KH_2PO_4, 1 $MgCl_2$, 10

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1. *Abbreviations used in this paper:* AVP, 8-arginine vasopressin; CCD, cortical collecting duct; DA, dopamine; DA_1, fenoldopam; DARPP-32, dopamine- and cAMP-regulated phosphoprotein, 32,000 M_r; dBcAMP, dibutyryl cAMP; DDA, 2'5'-dideoxyadenosine; FSK, forskolin; IP_{20}, protein kinase A inhibitory peptide; MTAL, medullary thick ascending limbs; PKA, protein kinase A; PKC, protein kinase C; PLA_2, phospholipase A_2.

Tris-HCl, 1 $CaCl_2$ at pH 7.4 with 0.05% collagenase (Type I; Sigma Chem. Co., St. Louis, MO), and 0.1% BSA. The kidney was removed and cut along the corticopapillary axis into pyramids, which were incubated in the same collagenase solution at 37°C for 7 min. After incubation, the pyramids were rinsed with cold modified HBSS (0.25 mM $CaCl_2$), and CCD segments were dissected freehand in the same solution at 4°C under stereomicroscopic observation. Isolated CCD segments were individually transferred to a concave bacteriological slide and photographed to determine their length.

Determination of Na-K-ATPase activity. Tubules were incubated in 1 μl HBSS (0.25 mM $CaCl_2$) supplemented with 5 mM glucose, 2 mM Na acetate, and 5 mM Na lactate, with or without agonists at 37°C for 15–30 min. Tubules were then permeabilized in a hypotonic medium (10 mM Tris, pH 7.4), followed by rapid freezing on dry ice. Total ATPase activity was determined after 15 min incubation at 37°C in a 1-μl droplet containing (mM) 50 NaCl, 5 KCl, 10 $MgCl_2$, 1 EGTA, 100 Tris-HCl, 10 Na_2ATP (grade II, vanadate-free; Sigma Chemical Co.) and [γ-^{32}P]ATP (Amersham, Arlington Heights, IL) in tracer amounts (~ 5 nCi/μl). Magnesium-dependent ATPase activity was determined in the same solution containing 4 mM ouabain. Phosphate liberated by the hydrolysis of [γ-^{32}P]ATP was separated by filtration through a Millipore filter (0.45 μm pore size) after adsorption of the unhydrolyzed nucleotide on activated charcoal (Sigma Chem. Co.), and the radioactivity was counted in a liquid scintillation spectrometer (Packard Instr. Co., Inc., United Technologies, Downers Grove, IL). Total and Mg-dependent ATPase activity were each determined on four or five replicate samples from individual animals, and calculated per millimeter tubule length. Na- and K-dependent, ouabain-inhibitable ATPase was taken as the difference between the means of each group of measurements, and thus represents a single datum point in each animal. To minimize variability between experiments, Na-K-ATPase activity was always measured in experimental and control tubules simultaneously.

Determination of cAMP content. cAMP content in isolated CCD was measured by the method of Torikai and Kurokawa (20), with minor modifications. In brief, tubules were preincubated for 5 min at 37°C in 20 μl supplemented HBSS in the presence of 3-isobutyl-1-methylxanthine (IBMX, 1.2 mM). 20 μl of the same solution with or without agonists was then added, and tubules were incubated at 37°C for 7 min, the time period found in preliminary experiments to yield maximum cAMP accumulation. The reaction was terminated by addition of 50 μl 10% trichloroacetic acid and rapid cooling on ice. The mixture was extracted three times with 0.8 ml of water-saturated ether, followed by evaporation under nitrogen. The extracts were stored at −20°C until assay. cAMP content was determined with a highly sensitive radioimmunoassay (Biomed. Technols., Inc., Stoughton, MA) after acetylation.

Materials. Fenoldopam was kindly provided by Dr. Michael Murphy, Department of Pharmacological and Physiological Sciences, University of Chicago. 2'5'-dideoxyadenosine (DDA) was purchased from Pharmacia LKB Biotechnology (Piscataway, NJ), and Sch 23390 from Res. Biochems. Inc. (Natick, MA). DA and all other chemicals were obtained from Sigma Chem. Co. Solutions of DA and fenoldopam were prepared each day in concentrated form (1 mg/ml) in slightly acidified double distilled water (one drop 1 N HCl/ml) and brought to pH 7.4 by dilution in the buffered media used for incubation. Forskolin was stored as a 5-mg/ml solution in dimethylsulfoxide. Stock solutions of 8-arginine vasopressin (AVP), arachidonic acid, DDA, and protein kinase A inhibitory peptide (IP_{20}) were stored at −20°C until use. dBcAMP and mepacrine were prepared fresh each day.

Statistics. Statistical analysis was done with one-way analysis of variance, followed by the Bonferroni correction for multiple comparisons. Results in text and figures are means±SE.

Results

Role of cAMP. DA and the DA_1 agonist fenoldopam (both 10^{-5} M) increased cAMP accumulation in CCD from 3.1±0.3 to 5.5±0.4 and 5.6±0.6 fmol · mm^{-1} · 7 min^{-1}, respectively (both, $P < 0.01$; Fig. 1). This moderate increase in cAMP is in accord with that found in renal tissue by others (7) and contrasts with the large (> 20-fold) increment measured by us in CCD in response to AVP and forskolin (not shown). The effect of either agonist was completely blocked by 10^{-4} M DDA, a potent inhibitor of adenylate cyclase (21, 22). As expected, Sch 23390 (10^{-3} M), a selective DA_1 antagonist, also inhibited completely the effect of DA on cAMP accumulation (Fig. 1), indicating that it is mediated via the DA_1 receptor.

To evaluate the role of cAMP-protein kinase A pathway in DA-mediated pump inhibition, we designed the following protocols. First, we determined whether the effect of DA on Na-K-ATPase activity in the CCD can be reproduced by other agonists that increase cell cAMP, and we chose several which do so by various mechanisms: receptor-mediated activation of adenylate cyclase by AVP, direct activation of adenylate cyclase by forskolin (23), and addition of exogenous cAMP using the membrane permeant analogue dBcAMP. As shown in Fig. 2, AVP (10^{-8} M), forskolin (10^{-5} M), and dBcAMP (10^{-3} M) each produced pump inhibition comparable to that of DA or fenoldopam (all, $P < 0.001$). We next examined the effects of inhibitors of the cAMP-PKA pathway on the pump inhibition by these agents. DDA completely blocked the pump inhibition by DA or fenoldopam (Fig. 3). IP_{20} (10^{-8} M), a selective inhibitor of PKA (24), also completely abolished the effect of DA or fenoldopam, as well as that of AVP, forskolin, and dBcAMP (Fig. 4). These results strongly suggest that DA, acting via its DA_1 receptor, inhibits Na-K-ATPase activity in the CCD through a cAMP- and PKA-dependent mechanism.

Role of PLA_2. In a separate group of experiments, we examined the effect of mepacrine, an inhibitor of PLA_2, on the cAMP-PKA pathway-mediated pump inhibition. Mepacrine (10^{-5} M) also completely blocked the pump inhibition by DA or fenoldopam, as well as that produced by AVP, forskolin, or dBcAMP (Fig. 5). These observations suggest that the cAMP-PKA pathway may be involved in PLA_2 activation in CCD, and that the latter could play a role in the DA_1 receptor-mediated pump inhibition in this segment, probably by increasing arachidonic acid release. As shown in Fig. 6, exogenous arachidonic acid inhibited Na-K-ATPase activity in dose-dependent manner (maximum at 10^{-5} M), further supporting the hypothesis that PLA_2 activation participates in pump regulation.

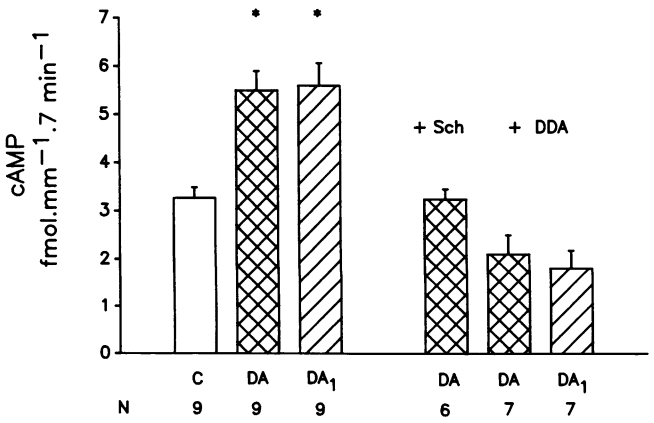

Figure 1. Effect of dopamine (*DA*) or fenoldopam (*DA_1*) on cAMP accumulation in CCD. Tubules were exposed to DA or fenoldopam (both 10^{-5} M) in the presence or absence of 2',5'-dideoxyadenosine (*DDA*, 10^{-4} M) or Sch 23390 (*Sch*, 10^{-3} M). *$P < 0.01$ vs. control (*C*).

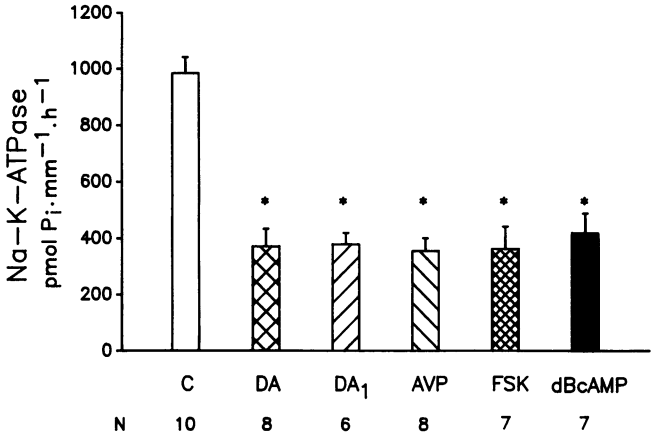

Figure 2. Modulation of Na-K-ATPase activity in CCD by agents that increase cell cAMP. Tubules were exposed to DA or fenoldopam (DA_1) (both 10^{-5} M), or to AVP (10^{-8} M) for 30 min, and to forskolin (*FSK*, 10^{-5} M) or dBcAMP (10^{-3} M) for 15 min. $^*P < 0.001$ vs. control.

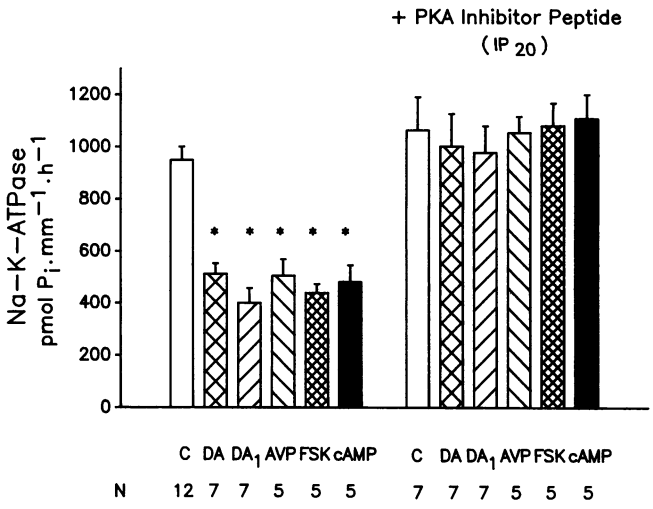

Figure 4. Effect of protein kinase A inhibitory peptide (IP_{20}) on Na-K-ATPase inhibition by cAMP agonists in CCD. Tubules were incubated with DA, fenoldopam (both 10^{-5} M), AVP (10^{-8} M), forskolin (*FSK*, 10^{-5} M), or dBcAMP (*cAMP*, 10^{-3} M) in the presence or absence of 10^{-8} M IP_{20}. $^*P < 0.001$ vs. control.

To evaluate how the cAMP-PKA pathway interacts with PLA_2, we used corticosterone as a representative glucocorticoid because it occurs naturally in the rat. Glucocorticoids induce the synthesis of a protein that inhibits PLA_2 (25, 26) and is inactivated by PKA (27), termed lipomodulin (27). Corticosterone alone (75 nM) produced a slight, but not statistically significant, stimulation of pump activity after 90 min incubation. When combined with DA, fenoldopam, forskolin, or dBcAMP, however, corticosterone completely prevented their inhibition of the pump (Fig. 7). Although indirect, these observations suggest that in CCD, cAMP-dependent PKA activation of PLA_2 might be due to inactivation of lipomodulin, presumably by its phosphorylation.

Discussion

The present study demonstrates that Na-K-ATPase inhibition by DA or the DA_1 agonist fenoldopam in the CCD was reproduced by agents that increase cAMP content by various mechanisms, and was abolished by inhibitors of adenylate cyclase or PKA, suggesting that the cAMP-PKA pathway mediates the action of DA on the pump in this segment. Mepacrine, an inhibitor of PLA_2, completely blocked the effect of DA or fenoldopam, as well as that of AVP, forskolin, or dBcAMP on Na-K-ATPase activity, and arachidonic acid produced a dose-dependent inhibition of the pump. These results suggest, in addition, that activation of PLA_2 participates in the cAMP-dependent pump regulation in CCD.

DA, acting via its DA_1 receptor, increases cAMP accumulation and inhibits Na-K-ATPase activity in PCT (3, 4). We recently observed similar actions of dopamine in the CCD as well (Takemoto, F., H. T. Cohen, T. Satoh, and A. I. Katz, manuscript submitted for publication), where we also demonstrated specific DA_1 binding sites (11). However, the role of cAMP in the mechanism of pump modulation in PCT and CCD might differ. Whereas interaction with both DA_1 and DA_2 receptors is required for pump inhibition in PCT (4), DA_1 receptor stimula-

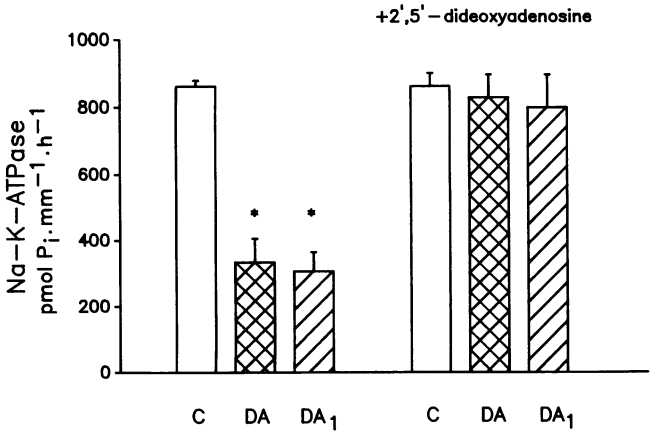

Figure 3. Effect of DDA on Na-K-ATPase inhibition by DA or fenoldopam (DA_1) in CCD. Tubules were incubated 30 min with DA or fenoldopam (10^{-5} M) in the presence or absence of DDA (10^{-4} M); $n = 6$ in each group. $^*P < 0.001$ vs. control.

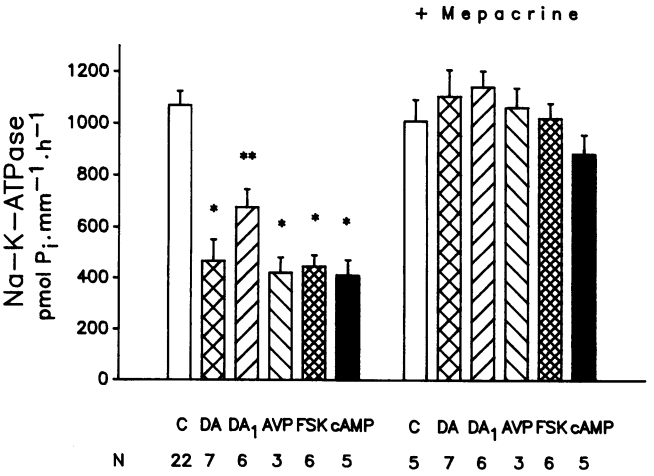

Figure 5. Effect of mepacrine on the pump inhibition by agonists that increase cAMP content in CCD. Tubules were incubated with DA, fenoldopam (DA_1), or AVP for 30 min, or with forskolin (*FSK*) or dBcAMP for 15 min (concentrations as in Fig. 2) in the presence or absence of mepacrine (10^{-5} M). $^*P < 0.001$; $^{**}P < 0.01$ vs. control.

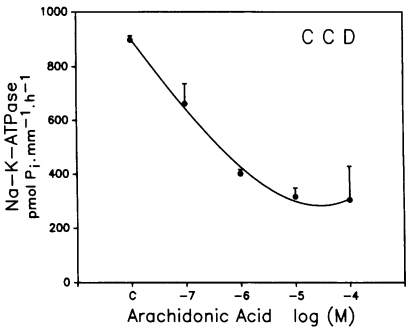

Figure 6. Dose-dependent inhibition of Na-K-ATPase activity in CCD by arachidonic acid. Tubules were incubated for 15 min. Each point represents the mean of measurements from 3 to 6 animals. $P < 0.001$ by analysis of variance (ANOVA).

tion alone can generate this effect in CCD (11). In addition, although neither dBcAMP nor forskolin alone inhibits the pump in PCT (4), the current study demonstrates that both these agents inhibited Na-K-ATPase activity in CCD (Fig. 2). Furthermore DDA, an agent that inhibits adenylate cyclase activity by acting at the internal inhibitory P site linked to the catalytic unit of the enzyme (21, 22), abolished cAMP accumulation and the pump inhibition by DA or fenoldopam (Figs. 1 and 3). IP_{20}, a potent 20-residue PKA inhibitory peptide (24), also prevented the effect of DA or fenoldopam (as well as of other cAMP agonists) on the CCD pump (Fig. 4). Taken together, these observations indicate that DA, acting on the DA_1 receptor, inhibits Na-K-ATPase activity in CCD through the cAMP-PKA pathway. The PKA inhibitory peptide also blocked the DA_1-mediated inhibition of Na-K-ATPase in MTAL (15) although, unlike in CCD in the present study, or in brain synaptosomes (13), the PKA inhibitor alone surprisingly reduced pump activity in this nephron segment.

The reason why cAMP alone can mediate the pump inhibition in CCD but not in PCT is unclear. In renal cortical membranes, derived chiefly from PCT, DA_1 receptors link not only to adenylate cyclase but also to phospholipase C (28, 29), which can subsequently stimulate protein kinase C (30). Bertorello and Aperia reported that activators of protein kinase C (PKC) inhibited Na-K-ATPase activity in PCT, and sphingosine, a PKC inhibitor, prevented the pump inhibition by DA (31). These observations suggest that the cellular mechanism of Na-K-ATPase inhibition in PCT is mediated by PKC (31), or involves both the cAMP-PKA and phospholipase C-PKC pathways. In CCD, in contrast, only the cAMP-PKA pathway appears to be involved in pump regulation. Besides the results reported in this paper, we have observed in preliminary experiments that several PKC activators (phorbol myristate acetate, phorbol dibutyrate, or dioctanoylglycerol) did not inhibit Na-K-ATPase activity, and that staurosporine, a relatively specific inhibitor of PKC, failed to block the pump inhibition by DA or fenoldopam in CCD (Satoh, T., H. T. Cohen, and A. I. Katz, unpublished observations).

The mechanism(s) whereby activation of PKA might regulate renal Na-K-ATPase is not clear. In the brain, cAMP inhibits Na-K-ATPase activity, but neither cAMP nor PKA interacts directly with this enzyme system (13), suggesting that other compounds generated through a cAMP-dependent mechanism mediate the inhibition. In MTAL, the phosphorylated form of DARPP-32 (dopamine- and cAMP-regulated phosphoprotein, 32,000 M_r), a potent inhibitor of protein phosphatase-1, has been reported to mediate the pump inhibition by DA. Because this agent did not inhibit the activity of a purified Na-K-ATPase preparation, it was suggested that it acts as an intracellular third messenger (15, 32). However, DARPP-32 is unlikely to play a role in CCD because DARPP-32 is concentrated in the MTAL, and its mRNA is not found in the renal cortex (15).

Our observations that mepacrine, an inhibitor of PLA_2, completely blocked the effect of DA or fenoldopam, as well as that of other cAMP agonists (Fig. 5), suggest that in CCD the cAMP-PKA pathway may mediate pump inhibition by stimulating PLA_2. Moreover, exogenous arachidonic acid inhibited pump activity in dose-dependent manner (Fig. 6), further supporting a role of PLA_2 in this phenomenon and raising the possibility that arachidonic acid products might be involved in cAMP-dependent pump regulation in CCD. A relationship between the cAMP-dependent pathway and arachidonate metabolism in Na-K-ATPase regulation has been proposed by Schwartzman et al. in a study of MTAL cells in which cAMP-dependent products of cytochrome P450-related arachidonate metabolites inhibited Na-K-ATPase activity (17). Hébert et al. recently reported that cAMP inhibited sodium transport in CCD through a cyclooxygenase-dependent mechanism, which also suggests activation of PLA_2 via the cAMP-dependent pathway (18).

How is activation of PKA linked to that of PLA_2? Based on studies in nonrenal tissue, Hirata (27) proposed that PLA_2 activity is regulated by lipomodulin, an ~ 40-kD phospholipase inhibitory protein that is phosphorylated by PKA, with loss of its ability to inhibit PLA_2 activity. Lipomodulin synthesis is enhanced by glucocorticoids (25–27), and Doucet (16) proposed that it may be responsible for the preservation of MTAL Na-K-ATPase activity during prolonged incubation. To evaluate a possible role of lipomodulin in our experiments, we used corticosterone, the natural glucocorticoid in the rat whose specific binding sites are concentrated in the CCD (33). Corticosterone prevented the pump inhibition by DA and fenoldopam, as well as by other cAMP agonists (Fig. 7), suggesting that lipomodulin might act as an intermediate regulator between PKA and PLA_2 in the cAMP-dependent pump inhibition observed in CCD. It is emphasized that the mode of pump regulation outlined

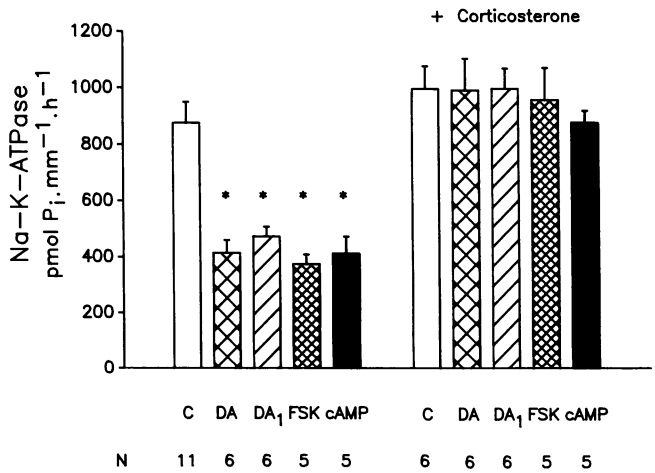

Figure 7. Effect of corticosterone on pump inhibition by DA, fenoldopam (DA_1), forskolin (*FSK*), or dibutyryl cAMP (*cAMP*) in CCD. Tubules were exposed to corticosterone (7.5×10^{-8} M) for 60 min, followed by incubation with the various agonists in the concentrations listed in Fig. 2 for 30 min in the presence or absence of the steroid. *$P < 0.001$ vs. control.

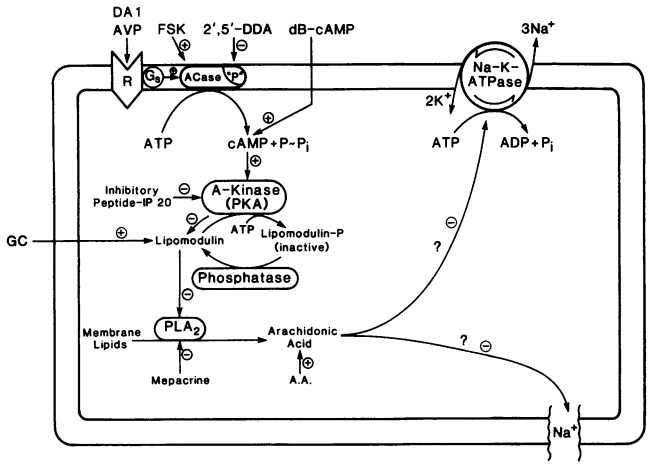

Figure 8. Schematic representation of the proposed cellular mechanisms of Na-K-ATPase regulation in the cortical collecting duct. GC, glucocorticoids; AA, arachidonic acid; other abbreviations are described in Figs. 1–4.

above applies only to the CCD, and does not exclude other mechanisms, e.g., a possible direct phosphorylation of Na-K-ATPase (or PLA_2) by PKA, which was not examined in this study.

The postulated mechanisms of Na-K-ATPase regulation in the CCD are summarized in Fig. 8. We propose that DA and other agonists that increase cell cAMP inhibit Na-K-ATPase activity in this nephron segment through the cAMP-PKA pathway and consequent stimulation of PLA_2 and arachidonic acid release, and that lipomodulin might provide the link between PKA and PLA_2. Cellular mechanisms after the activation of PLA_2, including the role of various arachidonate metabolites, and whether Na-K-ATPase regulation occurs by direct interaction with the pump or indirectly, e.g., through sodium entry, are currently being studied in our laboratory.

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References

1. Lee, M. R. 1982. Dopamine and the kidney. *Clin. Sci. (Lond.).* 62:439–448.

2. Siragy, H. M., R. A. Felder, N. L. Howell, R. L. Chevalier, M. J. Peach, and R. M. Carey. 1989. Evidence that intrarenal dopamine acts as a paracrine substance at the renal tubule. *Am. J. Physiol.* 257:F469–F477.

3. Aperia, A., A. Bertorello, and I. Seri. 1987. Dopamine causes inhibition of Na^+-K^+-ATPase activity in rat proximal convoluted tubule segments. *Am. J. Physiol.* 252:F39–F45.

4. Bertorello, A., and A. Aperia. 1990. Inhibition of proximal tubule Na^+-K^+-ATPase activity requires simultaneous activation of DA_1 and DA_2 receptors. *Am. J. Physiol.* 259:F924–F928.

5. Frederickson, E. D., T. Bradley, and L. I. Goldberg. 1985. Blockade of renal effects of dopamine in the dog by the DA_1 antagonist SCH 23390. *Am. J. Physiol.* 249:F236–F240.

6. Hughes, J. M., V. Ragsdale, R. A. Felder, R. L. Chevalier, B. King, and R. M. Carey. 1988. Diuresis and natriuresis during continuous dopamine-1 receptor stimulation. *Hypertension (Dallas).* 11(Suppl. I):I69–I74.

7. Felder, R. A., C. C. Felder, G. M. Eisner, and P. A. Jose. 1989. The dopamine receptor in adult and maturing kidney. *Am. J. Physiol.* 257:F315–F327.

8. Felder, R. A., M. Blecher, P. L. Calcagno, and P. A. Jose. 1984. Dopamine receptors in the proximal tubule of the rabbit. *Am. J. Physiol.* 247:F499–F505.

9. Huo, T., and D. P. Healy. 1989. Autoradiographic localization of dopamine DA_1 receptors in rat kidney with [^{3}H]Sch 23390. *Am. J. Physiol.* 257:F414–F423.

10. Kinoshita, S., E. H. Ohlstein, and R. A. Felder. 1990. Dopamine-1 receptors in rat proximal convoluted tubule: regulation by intrarenal dopamine. *Am. J. Physiol.* 258:F1068–F1074.

11. Takemoto, F., T. Satoh, H. T. Cohen, and A. I. Katz. 1991. Localization of dopamine-1 receptors along the microdissected rat nephron. *Pfluegers Arch. Eur. J. Physiol.* 419:243–248.

12. Tria, E., P. Luly, V. Tomasi, A. Trevisani, and O. Barnabei. 1974. Modulation by cyclic AMP in vitro of liver plasma membrane (Na^+-K^+)-ATPase and protein kinases. *Biochim. Biophys. Acta.* 343:297–306.

13. Lingham, R. B., and A. K. Sen. 1982. Regulation of rat brain (Na^+ + K^+)-ATPase activity by cyclic AMP. *Biochim. Biophys. Acta.* 688:475–485.

14. Tung, P., G. Pai, D. G. Johnson, R. Punzalan, and S. R. Levin. 1990. Relationships between adenylate cyclase and Na^+,K^+-ATPase in rat pancreatic islets. *J. Biol. Chem.* 265:3936–3939.

15. Meister, B., J. Fryckstedt, M. Schalling, R. Cortés, T. Hökfelt, A. Aperia, H. C. Hemmings, Jr., A. C. Nairn, M. Ehrlich, and P. Greengard. 1989. Dopamine- and cAMP-regulated phosphoprotein (DARPP-32) and dopamine DA_1 agonist-sensitive Na^+,K^+-ATPase in renal tubule cells. *Proc. Natl. Acad. Sci. USA.* 86:8068–8072.

16. Doucet, A. 1988. Multiple hormonal control of the Na/K-ATPase activity in the thick ascending limb. *Proc. Int. Congr. Nephrol.* 10:247–254.

17. Schwartzman, M., N. R. Ferreri, M. A. Carroll, E. Songu-Mize, and J. C. McGiff. 1985. Renal cytochrome P450-related arachidonate metabolite inhibits (Na^+ + K^+)ATPase. *Nature (Lond.).* 314:620–622.

18. Hébert, R. L., H. R. Jacobson, and M. D. Breyer. 1991. Prostaglandin E_2 inhibits sodium transport in rabbit cortical collecting duct by increasing intracellular calcium. *J. Clin. Invest.* 87:1992–1998.

19. Doucet, A., A. I. Katz, and F. Morel. 1979. Determination of Na-K-ATPase activity in single segments of the mammalian nephron. *Am. J. Physiol.* 237:F105–F113.

20. Torikai, S., and K. Kurokawa. 1983. Effect of PGE_2 on vasopressin-dependent cell cAMP in isolated single nephron segments. *Am. J. Physiol.* 245:F58–F66.

21. Londos, C., and J. Wolff. 1977. Two distinct adenosine-sensitive sites on adenylate cyclase. *Proc. Natl. Acad. Sci. USA.* 74:5482–5486.

22. Sadler, S. E., and J. L. Maller. 1983. Inhibition of *Xenopus* oocyte adenylate cyclase by progesterone and 2',5'-dideoxyadenosine is associated with slowing guanine nucleotide exchange. *J. Biol. Chem.* 258:7935–7941.

23. Seamon, K. B., W. Padgett, and J. W. Daly. 1981. Forskolin: Unique diterpene activator of adenylate cyclase in membranes and in intact cells. *Proc. Natl. Acad. Sci. USA.* 78:3363–3367.

24. Cheng, H.-C., B. E. Kemp, R. B. Pearson, A. J. Smith, L. Misconi, S. M. Van Patten, and D. A. Walsh. 1986. A potent synthetic peptide inhibitor of the cAMP-dependent protein kinase. *J. Biol. Chem.* 261:989–992.

25. Flower, R. J., and G. J. Blackwell. 1979. Anti-inflammatory steroids induce biosynthesis of a phospholipase A_2 inhibitor which prevents prostaglandin generation. *Nature (Lond.).* 278:456–459.

26. Blackwell, G. J., R. Carnuccio, M. Di Rosa, R. J. Flower, L. Parente, and P. Persico. 1980. Macrocortin: a polypeptide causing the anti-phospholipase effect of glucocorticoids. *Nature (Lond.).* 287:147–149.

27. Hirata, F. 1981. The regulation of lipomodulin, a phospholipase inhibitory protein, in rabbit neutrophils by phosphorylation. *J. Biol. Chem.* 256:7730–7733.

28. Felder, C. C., P. A. Jose, and J. Axelrod. 1989. The dopamine-1 agonist, SKF 82526, stimulates phospholipase-C activity independent of adenylate cyclase. *J. Pharmacol. Exp. Ther.* 248:171–175.

29. Felder, C. C., M. Blecher, and P. A. Jose. 1989. Dopamine-1-mediated stimulation of phospholipase C activity in rat renal cortical membranes. *J. Biol. Chem.* 264:8739–8745.

30. Majerus, P. W., T. M. Connolly, H. Deckmyn, T. S. Ross, T. E. Bross, H. Ishii, V. S. Bansal, and D. B. Wilson. 1986. The metabolism of phosphoinositide-derived messenger molecules. *Science (Wash. DC).* 234:1519–1526.

31. Bertorello, A., and A. Aperia. 1989. Na^+-K^+-ATPase is an effector protein for protein kinase C in renal proximal tubule cells. *Am. J. Physiol.* 256:F370–F373.

32. Aperia, A., J. Fryckstedt, L. Svensson, H. C. Hemmings, Jr., A. C. Nairn, and P. Greengard. Phosphorylated M_r 32,000 dopamine- and cAMP-regulated phosphoprotein inhibits Na^+,K^+-ATPase activity in renal tubule cells. *Proc. Natl. Acad. Sci. USA.* 88:2798–2801.

33. Kurt Lee, S.-M., M. A. Chekal, and A. I. Katz. 1983. Corticosterone binding sites along the rat nephron. *Am. J. Physiol.* 244:F504–F509.