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

II. Role of Eicosanoids

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Abstract

We recently reported a novel intracellular mechanism of renal Na-K-ATPase regulation by agents that increase cell cAMP, which involves protein kinase A-phospholipase A_2 and is mediated by one or more arachidonic acid metabolites (Satoh, T., H. T. Cohen, and A. I. Katz. 1992. *J. Clin. Invest.* 89:1496). The present studies were, therefore, designed to assess the role of eicosanoids in the modulation of Na-K-ATPase activity in the rat cortical collecting duct. The effect of various cAMP agonists (dopamine, fenoldopam, vasopressin, forskolin, and dibutyryl cAMP), which inhibited the pump to a similar extent (~ 50%), was independent of altered Na entry as it was elicited in the presence of amiloride or nystatin, or when NaCl was replaced with choline Cl. This effect was completely blocked by SKF 525A or ethoxyresorufin, two inhibitors of the cytochrome P450-dependent monooxygenase pathway, or by pretreating the animals with $CoCl_2$, which depletes cytochrome P450. Equimolar concentrations (10^{-7} M) of the cyclooxygenase inhibitors indomethacin or meclofenamate caused only a partial inhibition of the cAMP agonists' effect on the pump, whereas nordihydroguaiaretic acid or A 63162, two inhibitors of the lipoxygenase pathway, were without effect. Furthermore, two products of this pathway, leukotriene B_4 and leukotriene D_4, had no effect on Na-K-ATPase activity, and ICI 198615, a leukotriene receptor antagonist, did not alter pump inhibition by cAMP agonists. Several P450 monooxygenase arachidonic acid metabolites (5,6-epoxyeicosatrienoic acid; 11,12-epoxyeicosatrienoic acid; 11,12-dihydroxyeicosatrienoic acid; and 12(R)-hydroxyeicosatetraenoic acid) as well as PGE_2 inhibited the Na:K pump in dose-dependent manner, but the effect of PGE_2 was blocked when Na availability was altered, whereas that of 12(R)-HETE remained unchanged. We conclude that the cytochrome P450-monooxygenase pathway of the arachidonic acid cascade plays a major role in the modulation of Na:K pump activity by eicosanoids in the rat cortical collecting duct, and that products of the cyclooxygenase pathway may contribute to pump inhibition indirectly, by decreasing intracellular Na. (*J. Clin. Invest.* 1993. 91:409–415.) Key words: cortical collecting duct•cytochrome P450 monooxygenase • cyclooxygenase • lipoxygenase • leukotrienes

Introduction

Na-K-ATPase, the biochemical equivalent of the Na:K pump, plays a central role in epithelial sodium transport. Dopamine (DA),[1] a potent natriuretic hormone, decreases tubular sodium reabsorption (1, 2), due in part to the inhibition of Na-K-ATPase activity in the proximal convoluted tubule (3) and cortical collecting duct (CCD) (4). We recently reported that DA_1 receptor activation increased cAMP accumulation in the CCD, and that dopamine and other agents that increase cell cAMP in this segment, such as vasopressin, forskolin, or exogenous cAMP, inhibited Na-K-ATPase activity in a quantitatively comparable manner. This novel mechanism of Na:K pump modulation involves activation of protein kinase A (PKA), and subsequently of phospholipase A_2 (PLA_2) (5).

Arachidonic acid, which is released by PLA_2 activation, is metabolized to bioactive eicosanoids through either the cyclooxygenase, cytochrome P450-dependent monooxygenase, or the lipoxygenase pathways, one or more of which may mediate cAMP-dependent pump inhibition in the kidney. PGE_2, a cyclooxygenase product, inhibits renal Na-K-ATPase activity (6, 7) and mediates the decrease in CCD sodium reabsorption produced by cAMP (8). In addition, 11,12-dihydroxyeicosatrienoic acid (11,12-DHT), generated via the cytochrome P450-dependent monooxygenase pathway, also inhibits the renal Na:K pump, and its production is cAMP-dependent (9). Other metabolites of this pathway, such as 5,6-epoxyeicosatrienoic acid (5,6-EET) (10) and 12(R)-hydroxyeicosatetraenoic acid [12(R)-HETE] (11), inhibit renal Na-K-ATPase activity as well. In contrast, little is known about the effect of lipoxygenase products on the renal Na:K pump.

Besides the uncertainty regarding the pathway of arachidonic acid metabolism involved, it is also unclear whether the action of eicosanoids that mediate the cAMP-dependent inhibition is on the pump itself or indirect; e.g., by altering luminal Na entry. The purpose of this study was, therefore, to identify the arachidonic acid metabolic pathways involved in cAMP-dependent pump regulation in rat CCD and to elucidate the mechanism(s) underlying this phenomenon.

Methods

Microdissection. Kidneys were obtained from male Sprague-Dawley rats weighing 200–300 g that had free access to regular laboratory chow

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1. *Abbreviations used in this paper:* AA, arachidonic acid; AVP, arginine vasopressin; CCD, cortical collecting duct; DA, dopamine; DHT, dihydroxyeicosatrienoic acid; EET, epoxyeicosatrienoic acid; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroxyperoxyeicosatetraenoic acid; MTAL, medullary thick ascending limb; NDGA, nordihydroguaiaretic acid; PKA, protein kinase A; PLA_2, phospholipase A_2, SKF 525A, 2-diethylaminoethyl 2,2-diphenylvalerate hydrochloride.

and tap water. The procedure for tubule microdissection has been reported previously in detail (12). 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 (in mM) 137 NaCl, 5 KCl, 0.8 $MgCl_2$, 0.33 Na_2HPO_4, 0.44 KH_2PO_4, 1 $MgCl_2$, 10 Tris-HCl, 1 $CaCl_2$ at pH 7.4 with 400 U/ml collagenase (type I; Sigma Chemical Co., St. Louis, MO) and 0.05% 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 were individually transferred to a concave bacteriological slide and photographed to determine their length.

To obtain tubules with patent lumina (for experiments using amiloride or choline chloride), animals received by jugular vein infusion an agarose solution (1 ml/100 g body wt) containing (wt/vol) 0.45% NaCl, 10% mannitol, and 2% agarose (type IX; Sigma Chemical Co.) prewarmed to 37°C (13). At the onset of osmotic diuresis, the left ureter was ligated, and the left kidney was perfused with prewarmed collagenase solution containing 1% agarose (wt/vol). The renal pedicle was then ligated and the left kidney was removed and immersed in a cold HBSS solution for several minutes, thus converting the intraluminal agarose from liquid to gel phase, before processing the tubules as described above.

Determination of Na-K-ATPase activity. Incubations with or without agonists were carried out for 30 min (the time interval required for maximal Na-K-ATPase inhibition by dopamine) at 37°C in 1 μl of HBSS (0.25 mM $CaCl_2$), supplemented with 5 mM glucose, 5 mM lactate, and 2 mM acetate. In separate experiments, all sodium salts were eliminated and replaced with choline chloride and equimolar concentration of their respective acids. The procedures followed, with minor modifications, the protocols described previously in detail (12). Tubules were permeabilized in a hypotonic medium (10 mM Tris, pH 7.4) with or without agonists, and then subjected to rapid freezing on dry ice. Total ATPase activity was determined after a 15-min incubation at 37°C in a 1-μl droplet containing (in 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 Corp., 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 after adsorption of the unhydrolyzed nucleotide on activated charcoal (Sigma Chemical Co.), and the radioactivity was counted in a liquid scintillation spectrometer (Packard Instruments, United Technologies, Downers Grove, IL).

Total and Mg-dependent ATPase activity were each determined on four 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 was always measured in experimental and control tubules simultaneously.

Materials. Fenoldopam was provided by Dr. Michael Murphy, Department of Pharmacological and Physiological Sciences, University of Chicago. 2-diethylaminoethyl 2,2-diphenylvalerate hydrochloride (SKF 525A), *N*-hydroxy-*N*-[1-(4-phenylmethoxyphenyl) ethyl]acetamide (A 63162), and [1-[[2-methoxy-4-[[(phenylsulfonyl)amino]carbonyl]phenyl]methyl]-1H-indazol-6-yl]-carbonic acid cyclopentyl ester (ICI 198615) were gifts from, respectively, SmithKline Beecham Pharmaceuticals (King of Prussia, PA); Abbott Laboratories (North Chicago, IL); and ICI Pharma (Wilmington, DE). Nordihydroguaiaretic acid (NDGA) was purchased from Aldrich Chemical Co. (Milwaukee, WI), meclofenamate from Calbiochem-Behring Corp., (San Diego, CA), and 5,6-EET, 11,12-EET, 11,12-DHT, 12(S)-HETE, 20(OH)-arachidonic acid, and leukotriene B_4 and D_4 from Cayman Chemical Co. Inc., (Ann Arbor, MI). All other chemicals were purchased from Sigma Chemical Co.

Dimethylsulfoxide was used as a solvent for forskolin, nystatin, ICI 198615, and A63162, whereas indomethacin, ethoxyresorufin, NDGA, the leukotrienes, and the P450 monooxygenase products were dissolved in ethanol. Final concentrations of DMSO and ethanol during incubations were < 0.1% and < 0.5%, respectively, and were shown not to influence Na-K-ATPase activity in preliminary experiments using these solvents alone.

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

Effect of cAMP agonists on Na-K-ATPase activity

Several experimental maneuvers that increase cAMP accumulation by different mechanisms caused inhibition of Na-K-ATPase activity: receptor-mediated activation of adenylate cyclase by DA (10^{-5} M), a DA_1 agonist, fenoldopam (10^{-5} M), or arginine vasopressin (AVP) (10^{-8} M); direct activation of adenylate cyclase by forskolin (10^{-5} M); and addition of exogenous cAMP using the membrane permeant analog dibutyryl cAMP (10^{-3} M) (Fig. 1, *A–C, left panel;* Table I).

To determine whether the effect of these cAMP agonists occurs through a direct interaction with the pump, or indirectly (e.g., by decreasing luminal Na entry) we evaluated their effects on Na-K-ATPase activity in several experimental conditions in which Na entry is altered. First, we used the Na channel

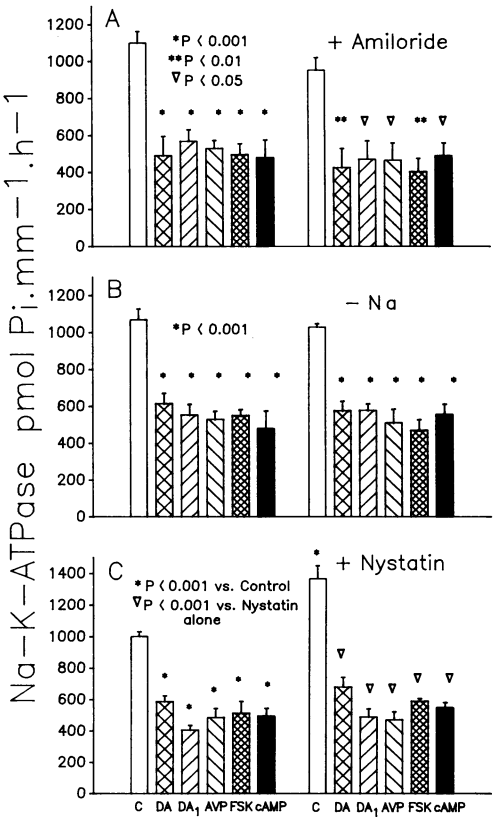

Figure 1. Effect of cAMP agonists on Na-K-ATPase activity in CCD in the presence or absence of agents that alter Na entry. *-Na*, NaCl replaced with choline Cl. Each bar represents the mean value from five to seven animals. □, Control; ⊠, DA; ▨, fenoldopam; ▧, AVP; ▩, forskolin; and ■, dibutyryl cAMP.

Table I. Effect of Various Modifiers on cAMP Agonists' Inhibition of Na-K-ATPase Activity in Rat CCD

Agonists	Additions							
	None	Ethoxyresorufin	None	Meclofenamate	None	A63162	None	ICI 198615
		10^{-7} M		10^{-5} M		10^{-6} M		10^{-6} M
None	965±60	1,053±130	977±63	897±86	1,082±33	964±104	1,130±50	1,030±67
Dopamine	476±67*	1,079±114	494±56*	710±59	505±48*	440±54‡	529±102‡	419±114‡
Fenoldopam	521±37‡	1,109±99	508±48*	694±74	501±50*	530±64§	469±70‡	536±86§
AVP	479±65*	1,051±169	525±28*	683±48	565±36*	448±78‡	403±108‡	473±97‡
Forskolin	427±99*	987±83	516±39*	701±44	517±66*	526±79§	507±111‡	482±89‡
dBcAMP	505±69‡	988±83	483±54*	703±32	562±54*	519±83§	463±96‡	408±46‡

* $P < 0.001$; ‡ $P < 0.01$; § $P < 0.05$. Na-K-ATPase activity is in pmol $P_i \cdot mm^{-1} \cdot h^{-1}$. There were five to seven animals in each group.

blocker amiloride at a concentration that inhibits Na entry but does not affect the pump itself (14). As shown in Fig. 1 *A*, amiloride (10^{-4} M) alone inhibited pump activity slightly but not significantly, and all cAMP agonists inhibited the pump even in its presence. Similarly, replacement of NaCl in the preincubation solution with choline Cl did not alter the effect of any cAMP agonist on Na-K-ATPase activity (Fig. 1 *B*). Last in this group of experiments, we determined the effect of nystatin, an ionophore that renders tubules permeable to Na so that it is no longer rate limiting for the pump. As expected, nystatin (50 μg/ml) alone significantly stimulated Na-K-ATPase. In the presence of nystatin, each cAMP agonist still inhibited pump activity to the same extent as in its absence (Fig. 1 *C*). These results indicate that the cAMP-dependent pump inhibition is not secondary to altered Na entry, and suggest that it is due mainly to interaction with the pump.

Role of eicosanoids in cAMP-dependent Na-K-ATPase inhibition

We reported elsewhere (5) that in the CCD, the effect of cAMP agonists on the Na:K pump involves activation of PKA and PLA_2 and could be reproduced with exogenous arachidonic acid. To assess the role of eicosanoid products in this phenomenon, we determined the effect of inhibiting each of the three major pathways of arachidonic acid metabolism on the cAMP-induced pump inhibition, as well as the effect of representative metabolites of each pathway on the CCD pump.

Role of the cytochrome P450-dependent monooxygenase pathway. We first determined the concentration dependence of various inhibitors of either the P450-dependent monooxygenase or cyclooxygenase pathway on the pump inhibition by the DA_1 agonist fenoldopam (10^{-5} M). Two inhibitors of cytochrome P450-dependent monooxygenase, SKF 525A (15) and ethoxyresorufin (16), completely blocked the effect of fenoldopam at concentrations ≥ 10^{-7} M (Fig. 2). In contrast, 10^{-7} M indomethacin, a cyclooxygenase inhibitor (17), caused only partial blockade, and near-complete elimination of the effect of fenoldopam required indomethacin concentrations of 10^{-6} M or greater (Fig. 2). Similarly, meclofenamate, another inhibitor of cyclooxygenase (17), did not block completely the fenoldopam effect, even at 10^{-5} M. (Fig. 2).

To determine whether cytochrome P450-dependent monooxygenase blockade affects the action of agents other than fenoldopam, we next determined the effect of low concentrations of SKF 525A or ethoxyresorufin on the inhibition of Na-K-ATPase by other cAMP agonists. SKF 525A (Fig. 3) or ethoxyresorufin (Table I), both 10^{-7} M, completely blocked the cAMP-mediated pump inhibition, strongly suggesting that cAMP-dependent pump regulation in CCD is mediated by metabolites of the cytochrome P450-dependent monooxygenase pathway. This postulate was confirmed in experiments with rats pretreated with $CoCl_2$ (50 mg/kg per d for 2 d), which depletes cytochrome P450 (18). None of the cAMP agonists inhibited pump activity significantly in CCD segments from these animals (Fig. 4).

Several metabolites of the cytochrome P450-dependent monooxygenase pathway [5,6-EET, 11,12-EET, 11,12-DHT, and 12(R)-HETE] inhibited the pump activity in dose-dependent fashion (10^{-12}–10^{-7} M), whereas the inhibition by 10^{-7} M 20(OH)-AA was not statistically significant; the inactive enantiomer 12(S)-HETE was without effect (Fig. 5). Furthermore, 12(R)-HETE (10^{-7} M) inhibited Na-K-ATPase activity in the presence of amiloride (10^{-4} M) or nystatin (50 μg/ml), or in the absence of Na (Fig. 6), indicating that metabolites of the monooxygenase pathway mediate cAMP-dependent pump inhibition independently of Na entry, as did the cAMP agonists (Fig.

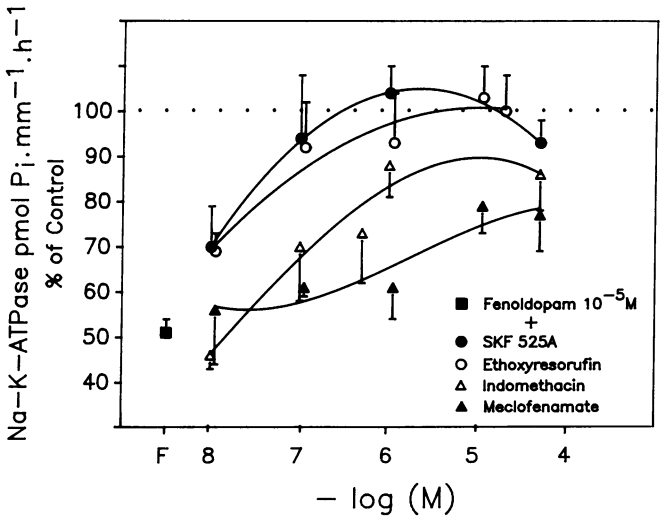

Figure 2. Inhibition of Na-K-ATPase activity in CCD by the DA_1 agonist fenoldopam (F), and reversal by inhibitors of cytochrome P450-dependent monooxygenase (SKF 525A, ethoxyresorufin) and cyclooxygenase (indomethacin, meclofenamate) pathways of arachidonic acid metabolism. Each point except fenoldopam alone ($n = 67$) represents the mean values from four to eight animals.

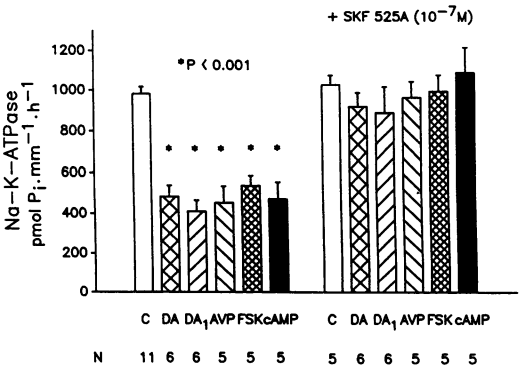

Figure 3. Effect of SKF 525A on the inhibition of Na-K-ATPase activity in CCD by cAMP agonists.

1). Taken together, these results provide compelling evidence that the cytochrome P450-dependent monooxygenase pathway plays an important role in Na:K pump inhibition by cAMP agonists, and that this effect probably occurs via a direct interaction with the pump.

Role of the cyclooxygenase pathway. Because cyclooxygenase inhibitors also altered the effect of cAMP agonists on the pump, we next evaluated the role of this pathway in cAMP-dependent pump regulation. PGE_2 inhibited Na-K-ATPase activity in dose-dependent manner (Fig. 5). Indomethacin at concentrations $\geq 10^{-6}$ M blocked the pump inhibition by fenoldopam (Fig. 2), as well as by the other cAMP agonists (data not shown). However, indomethacin blocked only partially the pump inhibition by all five cAMP agonists at 10^{-7} M (Fig. 7), a concentration at which the two inhibitors of cytochrome P450-dependent monooxygenase pathway were completely effective (Fig. 3 and Table I). In addition, the effect of meclofenamate on cAMP-dependent pump inhibition, although statistically significant, was also partial even at 10^{-5} M (Table I), the maximal concentration chosen on the basis of the dose-response curve in Fig. 2. Furthermore, PGE_2 (10^{-8} M) did not inhibit Na-K-ATPase activity in the presence of amiloride or nystatin, or in the absence of Na (Fig. 6). These results suggest that the cyclooxygenase pathway probably also plays a role in pump regulation, but that its action is on luminal Na entry rather than on the pump. That different mechanisms are involved in pump regulation by the cytochrome P450-dependent monooxygenase and cyclooxygenase pathways is further supported by

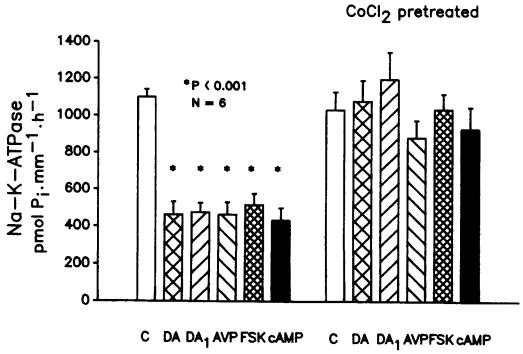

Figure 4. Effect of animals' pretreatment with $CoCl_2$ on the cAMP-induced inhibition of Na-K-ATPase activity in CCD.

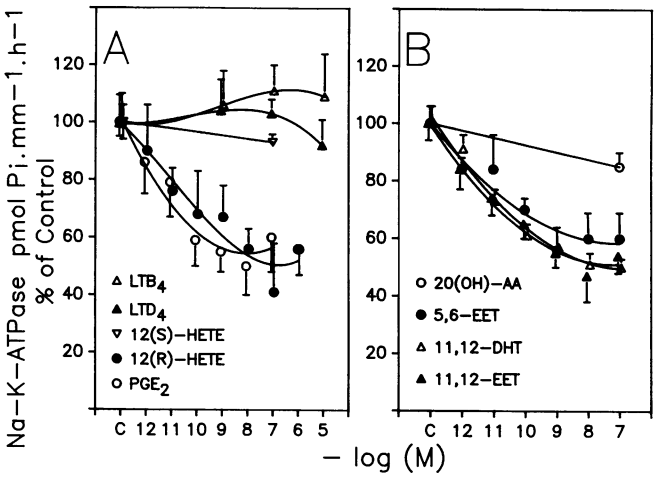

Figure 5. Effect of products of the three major pathways of arachidonic acid metabolism on Na-K-ATPase activity in CCD. Each point represents the mean value from four to eight animals.

the additive inhibitory effects of 12(R)-HETE and PGE_2 on the pump (Table II).

Role of the lipoxygenase pathway. Finally, we determined the role of the lipoxygenase pathway in cAMP-dependent pump inhibition. All five cAMP agonists inhibited pump activ-

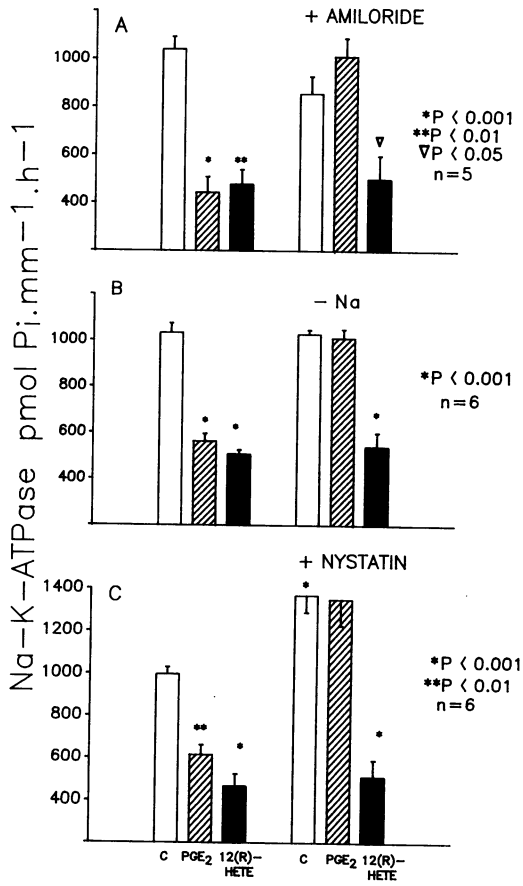

Figure 6. Inhibition of Na-K-ATPase activity in CCD by PGE_2 (10^{-8} M) and 12(R)-HETE (10^{-7} M), and its modification by agents that alter Na entry. □, Control, ▨, PGE_2; and ■, 12(R)-HETE.

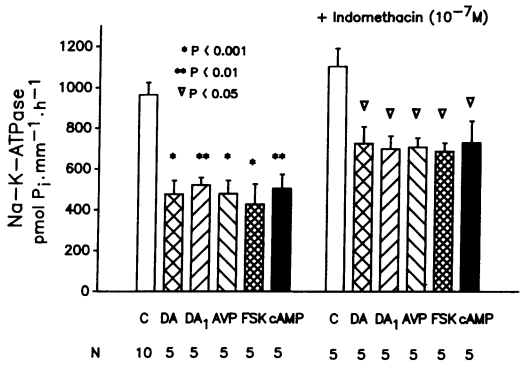

Figure 7. Effect of indomethacin on the inhibition of Na-K-ATPase activity in CCD by cAMP agonists.

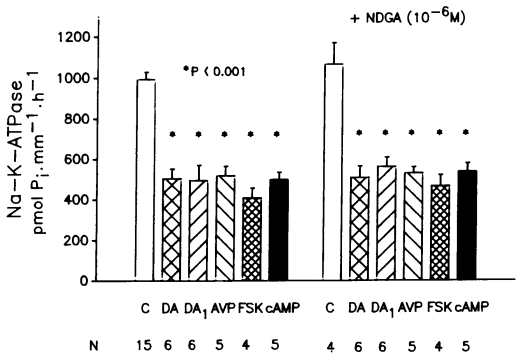

Figure 8. Effect of NDGA on the inhibition of Na-K-ATPase activity in CCD by cAMP agonists.

ity even in the presence of two potent lipoxygenase inhibitors: NDGA, 10^{-6} M (19) (Fig. 8), or A 63162, 10^{-6} M (20) (Table I), suggesting that the lipoxygenase pathway is not involved in cAMP-dependent pump regulation. Furthermore, two products of this pathway, leukotriene B_4 (LTB_4) and leukotriene D_4 (LTD_4), had no effect on Na-K-ATPase activity at concentrations up to 10^{-5} M (Fig. 5), and ICI 198615 (10^{-6} M), a potent and specific leukotriene receptor antagonist (21), did not block pump inhibition by cAMP agonists (Table I). These results argue against a role for the lipoxygenase pathway in the modulation of Na-K-ATPase in the CCD.

Discussion

We recently reported a novel intracellular mechanism of renal Na-K-ATPase regulation by agents that increase cell cAMP, which involves stimulation of protein kinase A and subsequently PLA_2, and is mediated by one or more arachidonic acid (AA) metabolites (5). The present study further delineates this mode of signal transduction in microdissected rat CCD. We assessed the role of the three major pathways of AA metabolism in cAMP-dependent pump regulation by using respective inhibitors and products of each. Because the specificity of such inhibitors is not entirely clear and is likely affected by the concentrations used, we chose two inhibitors of each pathway and determined the dose dependence of their effect, as well as that of representative products of arachidonate metabolism. Our observations indicate that both the cytochrome P450-dependent monooxygenase and cyclooxygenase (but not the lipoxygenase) pathways participate in cAMP-dependent Na-K-ATPase regulation. The mode of action of metabolites of these two pathways in the CCD differs, however. Products of cytochrome P450-dependent monooxygenase appear to interact with the Na:K pump, whereas cyclooxygenase products probably regulate the pump indirectly by altering luminal permeability for Na. Fig. 9 represents our proposed scheme for intracellular signaling in cAMP-dependent Na-K-ATPase regulation in CCD.

Table II. Inhibition of Na-K-ATPase Activity in Rat CCD by PGE_2 and 12(R)-HETE

	n	Na-K-ATPase	*P* (vs. control)	*P* (vs. both)
		pmol·mm^{-1}·h^{-1}		
Control	7	1,019±74	—	<0.001
PGE_2 (10^{-8} M)	7	596±44	<0.01	<0.02
12(R)-HETE (10^{-7} M)	7	560±26	<0.001	=0.03
PGE_2 + 12(R)-HETE	7	347±80	<0.001	—

AA can be metabolized by the cytochrome P450-dependent system to oxygenated products, such as HETE isomers, EETs, or their hydrolysis products, DHTs in the renal cortex (22), the CCD (23), and the outer medulla (24). In addition, PG synthesis by the cyclooxygenase pathway, a major product of which is PGE_2, has been demonstrated in the collecting duct (25). Products of either the cyclooxygenase or cytochrome P450 pathway have been reported to inhibit, directly or indirectly, renal Na-K-ATPase: PGE_2 inhibits the Na:K pump in the medullary thick ascending limb (MTAL) (7, 26), CCD (27, 28), and inner medullary collecting duct (6, 29, 30). Similarly, 5,6-EET inhibits the pump in proximal tubules (10); 11,12-DHT (9), 20-HETE, and 20-carboxy AA (31) do so in MTAL; and 12(R)-HETE inhibits purified renal cortical Na-K-ATPase (11). 12(R)-HETE, a product of the cytochrome P450-dependent monooxygenase pathway (32), is probably generated through 11,12-EET and/or 11,12-DHT (33). Although 12(R)-HETE has not been identified in the kidney, these putative precursors have been found there (9, 18, 22). Finally, both the cyclooxygenase- and cytochrome P450-dependent monooxygenase pathways mediate cAMP-induced effects on renal transport: In rabbit CCD, cAMP inhibits Na reabsorption by acting through the cyclooxygenase pathway (8), and 11,12-DHT generated by a cAMP-dependent mechanism inhibits Na-K-ATPase activity in MTAL (9). Our results are in agreement with these observations. The mechanism by which the cAMP-dependent pathway links to eicosanoid generation, however, is still not fully understood. We recently reported that in the CCD, lipomodulin might act as an intermediate regulator between PKA and PLA_2 (5). Another possible mechanism is nonspecific activation of PLA_2 (34) by cAMP-dependent intracellular Ca^{++} increase: Breyer has shown that in the rabbit CCD, cAMP raises intracellular Ca^{++} concentration, which contributes to the inhibition of sodium absorption by stimulating the basolateral Na^+/Ca^{++} exchanger (35).

In the present study, several P450-monooxygenase metabolites, as well as PGE_2, inhibited Na-K-ATPase activity in CCD in dose-dependent fashion (Fig. 5), and their maximally inhibitory concentrations (10^{-8} M–10^{-7} M), were considerably lower than those in previous reports (6, 32). SKF 525A (10^{-7} M), an

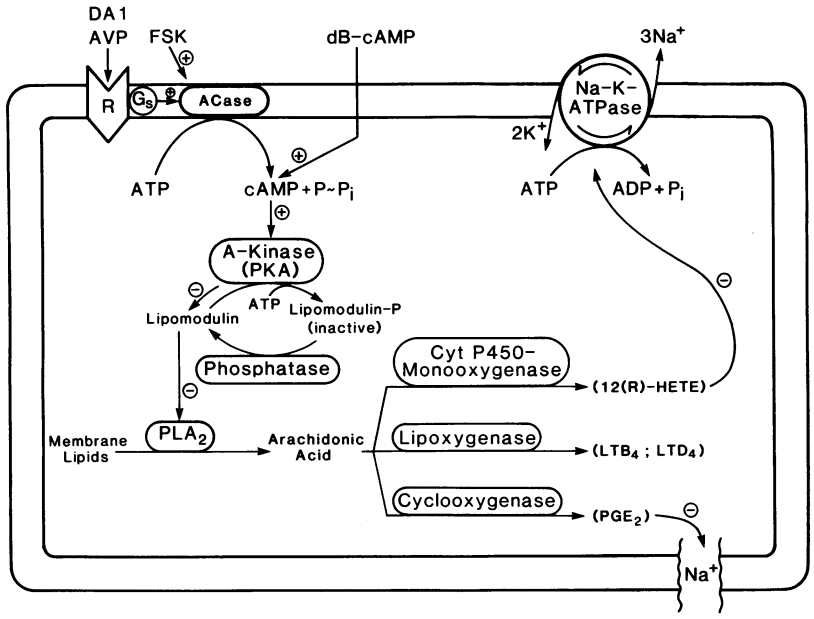

Figure 9. Schematic representation of the proposed cellular mechanisms of Na-K-ATPase regulation in the cortical collecting duct. Abbreviations are defined in the legend to Fig. 1 and in the text.

inhibitor of the cytochrome P450-dependent monooxygenase pathway, completely abolished the pump inhibition by fenoldopam, whereas the same concentration of indomethacin, an inhibitor of the cyclooxygenase pathway, was only partially effective; near complete inhibition was observed at $\geq 10^{-6}$ M (Fig. 5). SKF 525A at 10^{-7} M completely blocked the pump inhibition of each cAMP agonist tested (Fig. 3). In contrast, the effect of 10^{-7} M indomethacin on pump inhibition was only partial, and the agonists still inhibited Na-K-ATPase activity significantly (Fig. 7). These results indicate that both the cyclooxygenase- and cytochrome P450-dependent monooxygenase pathways participate in cAMP-dependent pump regulation in CCD, and suggest that the latter may play a more prominent role in this process. The monooxygenase pathway has also been implicated in the inhibition of MTAL Na-K-ATPase by AVP or AA (36).

The importance of cytochrome P450-dependent monooxygenase metabolites in cAMP-dependent Na-K-ATPase regulation, strongly suggested in this study by the dose-dependent inhibition of the pump elicited with several renal endogenous products of this pathway (Fig. 5), is further underscored by the following observations: First, depletion of cytochrome P450 by pretreating rats with $CoCl_2$ (18, 31) completely blocked the effect of the cAMP agonists (Fig. 4). In addition, ethoxyresorufin, a specific inhibitor of cytochrome P450-dependent monooxygenase that does not affect cyclooxygenase or lipoxygenase (16), at 10^7 M completely abolished the pump inhibition by all cAMP agonists tested (Table I), and also exerted the same dose-dependent blockade of the fenoldopam effect as SKF 525A (Fig. 2). In contrast, the cyclooxygenase inhibitor meclofenamate prevented only partially the cAMP-dependent pump inhibition, even at 10^{-5} M (Fig. 2, Table I), suggesting a lesser role of the cyclooxygenase pathway in this mechanism. We note that indomethacin has been reported to inhibit cytochrome P450-dependent arachidonic acid metabolism too, although at a concentration higher than that required for cyclooxygenase inhibition (37). Furthermore, the renovascular actions of some cytochrome P450-derived arachidonate metabolites can be blocked by indomethacin (38, 39). Our observations might, therefore, be explained by a greater specificity of meclofenamate compared with indomethacin for the cyclooxygenase pathway, but such a conclusion remains speculative.

In this study, we showed that each cAMP agonist inhibited Na-K-ATPase activity even in the presence of amiloride (10^{-4} M) or nystatin (50 μg/ml), or in the absence of Na (Fig. 1), suggesting that cAMP-dependent pump inhibition is independent of Na entry, and therefore, that it might involve a direct interaction with Na-K-ATPase. 12(R)-HETE, a representative product of the cytochrome P450-dependent monooxygenase pathway, also inhibited pump activity under these conditions of altered luminal Na entry, whereas the cyclooxygenase product PGE_2 did not (Fig. 6). These observations lend additional support to the notion that the cytochrome P450-dependent monooxygenase pathway mediates Na-K-ATPase inhibition as the cAMP agonists did (i.e., mainly by interacting with the pump), whereas the cyclooxygenase pathway seems to modulate the pump indirectly by reducing luminal Na entry. Marver (28) reported that PGE_2 did not inhibit CCD Na-K-ATPase in the presence of monensin, a Na ionophore, and Cordova et al. (27) found no direct effect on Na-K-ATPase when PGE_2 was added to CCD broken cell assays. Our results are consistent with these observations, although other investigators have suggested that the PGE_2 effect on Na-K-ATPase is independent of Na entry (7). The present study demonstrates that the combined effects of 12(R)-HETE and PGE_2 on the pump are additive (Table II), again suggesting different mechanisms of renal Na-K-ATPase regulation by these eicosanoids.

Last, lipoxygenase converts AA to the hydroxyperoxyeicosatetraenoic acids (HPETEs), which can then be converted to their corresponding (S)hydroxyeicosatetraenoic acids (HETEs) (17). The 5-lipoxygenase pathway generates through 5-HPETE the important leukotrienes, LTA_4 through LTE_4 (17), although little evidence support their synthesis in the kidney. Whether produced in renal or nonrenal cells, such lipoxygenase products could affect tubular sodium transport, as LTD_4 increases sodium channel activity in A6 cells (40) and various HPETEs inhibit purified Na-K-ATPase activity from the renal medulla (41). In the present study, however, all cAMP agonists inhib-

ited the pump activity even in the presence of 10^{-6} NDGA or 10^{-6} M A63162, both lipoxygenase inhibitors (Fig. 8 and Table I), suggesting that the lipoxygenase pathway is not involved in cAMP-dependent pump regulation in CCD. Moreover, two biologically active leukotrienes, LTB_4 and LTD_4, did not alter pump activity (Fig. 5), and ICI 198615 (10^{-6} M), a leukotriene receptor antagonist, did not block cAMP-dependent pump inhibition (Table I), providing further evidence that the lipoxygenase pathway does not participate in cAMP-dependent pump inhibition in CCD.

In summary, we propose that arachidonic acid metabolites of the cytochrome P450-dependent monooxygenase pathway play a central role in cAMP-dependent pump regulation in the CCD by direct interaction with the pump, and that the cyclooxygenase pathway contributes to cAMP-dependent Na-K-ATPase inhibition by limiting luminal Na permeability. The molecular mechanisms involved in the pump effect of the former remain to be elucidated.

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