ATP-Dependent Renal H^+ Translocation: Regional Localization, Kinetic Characteristics, and Chloride Dependence (43471)

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Abstract. **We characterized Mg^{2+}-dependent ATPase activity in membranes from the renal cortex, the outer and inner stripes of the outer medulla, and papillary vesicles. In all regions, there was Mg^{2+}-dependent ATPase activity that was resistant to oligomycin and vanadate and sensitive to *N,N'*-dicyclohexylcarbodiimide (DCCD), *N*-ethylmaleimide, and filipin. DCCD-Sensitive Mg^{2+}-ATPase activity was highest in the inner stripe of the outer medulla and lowest in the cortex, with intermediate values in the outer stripe of the outer medulla and papilla. The K_m for ATP, however, was similar among the different regions of the kidney. DCCD-Sensitive Mg^{2+}-ATPase activity was critically dependent upon chloride with K_m for Cl^- in the range of 2–5 m*M*. In the presence of ATP, this ATPase was capable of H^+ translocation, as assessed by acridine orange quenching. Inhibitors of ATPase activity prevented H^+ translocation, which suggests that the Mg^{2+}-ATPase represents, at least in part, an H^+-ATPase. H^+ transport was likewise critically dependent upon chloride, with similar K_m. The effect of chloride on H^+ translocation was blocked by the chloride channel inhibitor, diphenylamine-2 carboxylic acid. In the absence of chloride, H^+ transport was abolished, but it could be partially restored by the creation of a favorable electric gradient by K^+ and valinomycin. These studies demonstrate that the renal H^+-ATPase exhibits different activities in various regions of the kidney. The ATPase activity and H^+ translocation are critically dependent upon the presence of chloride, which suggests that chloride influences H^+ translocation by dissipating the H^+ gradient and acting at the catalytic site of the ATPase.**

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In recent years, it has been recognized that urinary acidification in the distal nephron of the the kidney and in analogs of the distal nephron is mediated by a H^+-ATPase (1–11). The renal H^+-ATPase is similar to other vacuolar H^+-ATPases (12–27). The renal H^+-ATPase is sensitive to *N*-ethylmaleimide (NEM) and *N,N'*-dicyclohexylcarbodiimide (DCCD), but resistant to oligomycin, and it is capable of H^+ translocation in the presence of ATP. ATP-dependent H^+ translocation and NEM-DCCD-sensitive H^+-ATPase was initially characterized in membranes of the renal medulla (5), but recent studies utilizing immunocytochemistry techniques have localized this H^+-ATPase in other nephron segments (28). Studies in microdissected nephron segments and in membranes prepared from renal cortex and medulla, have partially delineated the kinetic characteristics of the NEM-DCCD-sensitive ATPase that is thought to represent the H^+-ATPase (1). Studies in microdissected nephron segments, however, are limited because H^+ transport cannot be measured accurately and, hence, no correlation with ATPase activity can be done. The studies in membrane vesicles did not characterize the ATPase in membranes from different renal regions. H^+-ATPase in other tissues exhibit different sensitivities to anions; in some tissues, the H^+-ATPase is critically dependent upon chloride, whereas in others, this enzyme does not seem to be affected by the anion

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composition (12–19). Limited information is available about ATP-dependent H^+ translocation and its chloride dependence in various regions of the kidney that are important in urinary acidification. In the present study, we studied the kinetic characteristics of ATP-dependent H^+ translocation in various regions of the kidney, characterized its dependence upon chloride, and also characterized the kinetic characteristics of DCCD-sensitive Mg^{2+}-ATPase activity.

Methods

Membrane Vesicle Preparation. Membrane vesicles from different renal regions were prepared from several sets of six New Zealand White male rabbits each weighing 5–6 lb. Kidneys were dissected as described previously (29). In brief, each kidney was cut longitudinally into halves in a plane passing through the poles and hilum. Then, each half was cut transversely into 3–5-mm strips. Each layer was then separated, starting from the papilla and then dissecting the inner and outer stripes of outer medulla and the cortex. Luminal membrane vesicles from the cortex and outer stripe of the outer medulla (OSOM) were prepared by differential centrifugation and ionic precipitation techniques, as described by Sheikh *et al.* (30). Microscopic studies done on tissue samples of the OSOM showed that this layer was 95% free of cortical contamination. Briefly, tissues from either the cortex or the OSOM were suspended in 1:5 (w/v) of homogenizing medium A (300 m*M* sucrose, 25 m*M* Hepes-Tris [pH 7.00], and 0.2 m*M* phenylmethylsulfonyl fluoride), minced, and homogenized 30 times in a Teflon glass homogenizer. The homogenate was spun at 1,080*g* for 10 min. The supernatant was then spun at 12,100*g* for another 10 min. The resulting supernatant and the light fluffy layer were again spun at 38,000*g* for 30 min. The light fluffy layer was resuspended in medium B (300 m*M* sucrose, 25 m*M* Hepes-Tris [pH 7.00], 0.2 m*M* phenylmethylsulfonyl fluoride, 2.5 m*M* $MgCl_2$, and 2.5 m*M* $CaCl_2$) and was kept in the cold room overnight with constant stirring followed by centrifugation at 1,475*g* × 15 min. The supernatant was then spun at 48,500*g* for 30 min and the final pellet was resuspended in medium A to bring the protein concentration to 6–8 mg/ml.

Membranes from the inner stripe of the outer medulla (ISOM) were prepared using methods previously described by our laboratory (29). In brief, medullary tissues were suspended in 1:6 (w/v) of buffer containing 250 m*M* sucrose, 5 m*M* Tris-HCL (pH 7.40), 1 m*M* $NaHCO_3$, 1 m*M* dithiothreitol, and 1 m*M* EGTA and were minced and homogenized 30 times in a Teflon glass homogenizer. The resulting homogenate was then spun at 6,000*g* for 15 min. The supernatant was collected and centrifuged at 38,000*g* for 1 hr. The light fluffy layer of the pellet was suspended in homogenizing buffer and placed on top of a three-layer sucrose gradient (15%, 25%, and 40% wt/wt sucrose) that was spun for 16 hr at 100,000*g*. Membrane vesicle fractions collected at 15–25% (F_1 fraction) and 25–40% (F_2 fraction) interfaces were spun at 38,000*g* for 1 hr.

Papillary plasma membranes were also prepared by the same technique as described above, but the sucrose gradient step was excluded because of inadequate yield. All prepared membrane vesicles were stored in aliquots at −90°C until they were used for transport studies.

Enzymatic Assays. Protein concentration was assayed by the method of Bradford (31), using protein-dye binding techniques, alkaline phosphatase was assayed by the method of Lansing *et al.* (32), γ-glutamyltransferase by the method of Orlowski and Meister (33), Na-K-ATPase by the method of Brodsky *et al.* (34), and succinate dehydrogenase by the ferricyanide method of King (35).

DCCD-Sensitive H^+-ATPase activity was measured, as described previously (2), in the presence of ouabain (0.5 m*M*) to inhibit Na-K-ATPase, in the presence of oligomycin (10 μg/ml) to inhibit mitochondrial ATPase, and in the absence of Ca^{2+} to inhibit Ca^{2+}-ATPase. The oligomycin-sensitive H^+-ATPase assay was a modification of a DCCD-sensitive H^+-ATPase assay done in the absence or presence of oligomycin (7).

H^+-Transport Assay. H^+ Transport was assayed by acridine orange quenching as described previously (7). Briefly, membrane vesicles were preincubated in a buffer solution containing 150 m*M* KCl, 6 m*M* $MgCl_2$, 2 m*M* 2-[*N*-morpholina]ethanesulfonic acid, 2 m*M* Tris (pH 7.00). Assays were done in the presence of 10 μg/ml of oligomycin, 0.5 m*M* ouabain, and 6 μ*M* acridine orange. Protein (300 μg) was added to a total reaction mixture of 2 ml. H^+ Transport was started by adding 1 m*M* ATP once a steady baseline was achieved. Then, 1 μ*M* nigericin was added to dissipate the quenching.

Materials

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO), except diphenylamine-2 carboxylic acid (ICN Biomedicals, Inc., Costa Mesa, CA) and acridine orange (Eastman Kodak, Rochester, NY).

Results

Enzymatic Enrichment Factor in Membranes from Various Renal Regions. Table I shows the enrichment factor for the different enzymes in membrane vesicles prepared from various regions of the kidney. Membranes prepared from the renal cortex showed enrichment in alkaline phosphatase and γ-glutamyltransferase without basolateral and mitochondrial contamination, as assessed by the lack of enrichment in Na-K-ATPase and succinate dehydrogenase, respec-

Table I. Enrichment Factor of Various Enzymes in Membrane Vesicles Prepared from Cortex, Outer and Inner Stripes of Outer Medulla, and Papilla[a]

	Cortex	OSOM	ISOM		Papilla
			15–25% Fraction	25–40% Fraction	
Alkaline phosphatase	9.3 ± 0.6	10.1 ± 0.5	3.6 ± 1.0	5.8 ± 1.7	—
γ-Glutamyltransferase	8.8 ± 0.8	8.8 ± 0.8	3.4 ± 0.8	4.9 ± 0.6	—
Na-K-ATPase	0.4 ± 0.1	0.4 ± 0.1	2.1 ± 0.5	9.1 ± 0.9	2.8 ± 0.7
Succinate dehydrogenase	1.1 ± 0.1	1.8 ± 0.2	2.1 ± 0.2	1.9 ± 0.1	1.5 ± 0.2
DCCD-sensitive Mg^{2+}-ATPase	3.0 ± 0.7	9.1 ± 0.9	25.3 ± 2.0	4.9 ± 0.7	7.4 ± 1.2

[a] The membranes used for determination of marker enzymes were from the same batch of membranes used in a previous study for this laboratory (Ref. 29) (n = 5–6 in each group). Values are means ± SE.

tively. Likewise, membranes prepared from the OSOM were also enriched in alkaline phosphatase and γ-glutamyltransferase without basolateral and mitochondrial contamination.

Membranes from the ISOM were divided into two fractions: F_1 represents the fraction collected at the 15–25% interface of the sucrose gradient and F_2 represents the fraction collected at the 25–40% interface. Although there is no classic marker enzyme for these membranes, the F_1 fraction was enriched in alkaline phosphatase and γ-glutamyltransferase, with minimal enrichment in Na-K-ATPase and succinate dehydrogenase. In contrast, the F_2 fraction was enriched 9-fold in Na-K-ATPase, which suggests that this fraction was likely from the basolateral membranes. The papillary membranes were a mixture of luminal and basolateral membranes, as suggested by the enrichment in DCCD-sensitive ATPase and in Na-K-ATPase activities. The results of the DCCD-sensitive ATPase are discussed below.

ATP-Dependent H^+ Transport in Various Renal Regions: Chloride Dependence and Kinetics. To demonstrate that these membranes from various regions of the kidney are capable of ATP H^+ translocation, we examined the effect of ATP addition on the quenching of acridine orange. The addition of ATP to membranes from various regions of the kidney resulted in quenching of acridine orange that could be dissipated by nigericin, indicating the generation of H^+ gradient (Fig. 1). Not shown is the fact that filipin, DCCD, and NEM abolished completely the quenching of acridine orange. The above data strongly suggest that H^+ translocation is mediated by a H^+-ATPase.

Figure 2 shows that ATP addition did not elicit quenching of acridine orange fluorescence in vesicles from ISOM loaded with K gluconate and suspended in equimolar amounts of K gluconate. In contrast, in vesicles loaded with KCl and suspended in the same amount of KCl, ATP addition elicited quenching of acridine orange fluorescence that could be dissipated by nigericin. In vesicles loaded with K gluconate and suspended in K gluconate with 10-fold K gradient (in

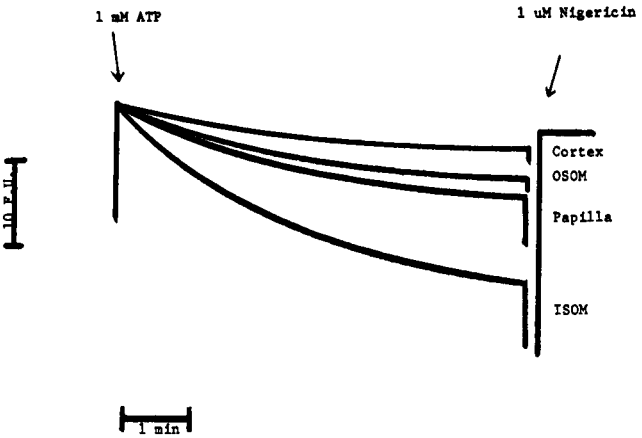

Figure 1. Quenching of acridine orange by luminal membrane vesicles from the cortex, outer stripe of outer medulla, inner stripe of outer medulla and plasma membrane vesicles from the papilla. Addition of nigericin dissipated the quenching.

> out), addition of ATP led to a small quenching of acridine orange. Addition of valinomycin to create a negative intracellular potential increased the quenching, although not to the level seen in presence of chloride. Figure 2 also shows that the effect of ATP to decrease acridine orange fluorescence is inhibited partially by diphenylamine-2-carboxylic acid, a chloride channel inhibitor. Similar qualitative results were obtained in vesicles from OSOM (Fig. 3) and from other regions of the kidney (not shown).

Figure 4 compares the effect of chloride on DCCD-sensitive Mg^{2+}-ATPase activity and on H^+ transport in membranes from different regions of the kidney. It is clear that in the absence of chloride, H^+ transport and ATPase activity were reduced by 50%. Increasing chloride concentration increased both H^+ transport and ATPase activity in ISOM (Fig. 4A), which were maximal at 20 mM chloride. The apparent K_m for chloride for H^+ transport and ATPase activity was in the range of 2–5 mM. Similar effects of chloride on H^+ transport and ATPase activity were observed in the papilla (Fig. 4B), OSOM (Fig. 4C), and cortex (Fig. 4D).

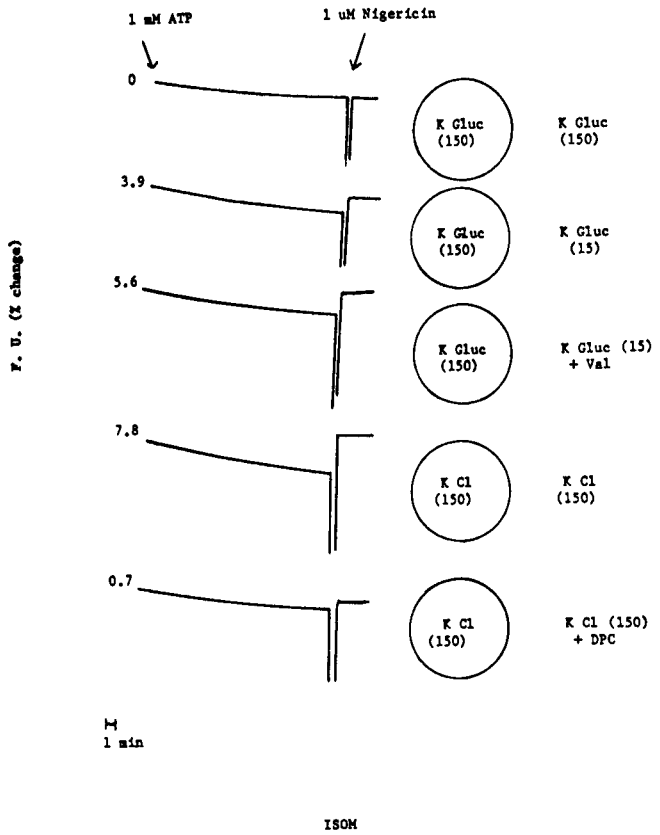

Figure 2. Quenching of acridine orange by membrane vesicles from the ISOM by addition of ATP. In presence of K gluconate in equal concentrations on both sides, there was no quenching of acridine orange (first tracing). In the presence of a K^+ gradient and in the presence of valinomycin, there was quenching of acridine orange, even though it was not the same level as that observed in the presence of chloride. The chloride channel inhibitor partially blocked the quenching of acridine orange.

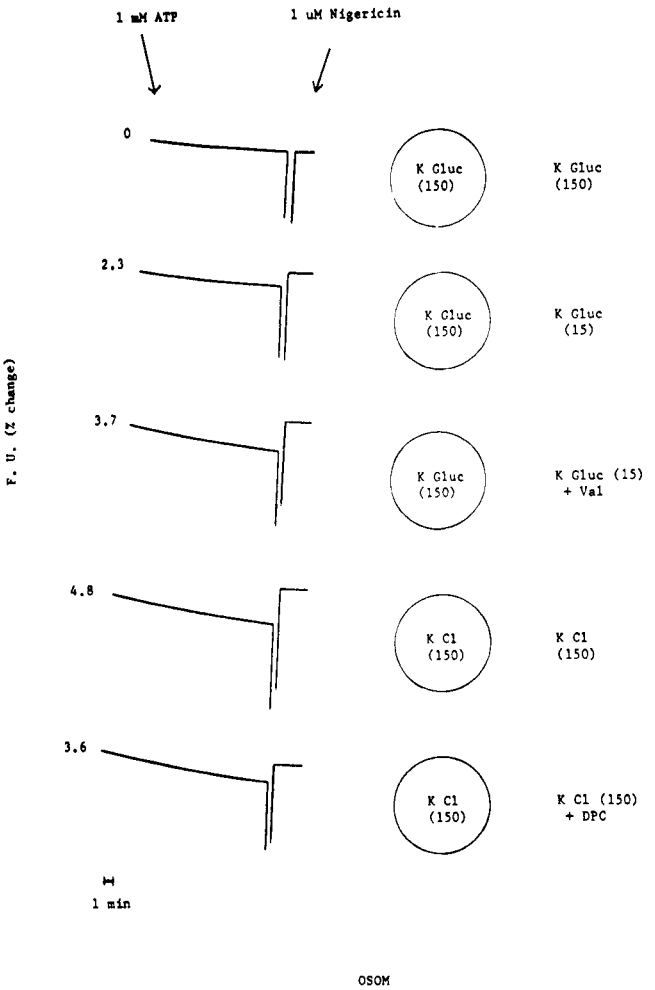

Figure 3. Quenching of acridine orange by luminal membrane vesicles from the outer stripe of outer medulla in the presence and absence of chloride.

DCCD-Sensitive Mg^{2+}-ATPase Activities in Various Regions of the Kidney, ATP, and Chloride Kinetics. We measured Mg^{2+}-ATPase activity in membranes prepared from various regions of the kidney. Mg^{2+}-ATPase activity was measured in the presence of ouabain to inhibit Na-K-ATPase, in the absence of calcium to inhibit Ca^{2+}-ATPase, in the presence of oligomycin to inhibit mitochondrial ATPase, and in the presence and absence of 200 μM DCCD. This ATPase is hereafter designated DCCD-sensitive ATPase. The ISOM 15–25% fraction had the highest activity (5.20 ± 1.24 nmol/μg protein/min), followed by the papilla (1.27 ± 0.05), OSOM (0.76 ± 0.04), and cortex (0.16 ± 0.02). Table I shows the enrichment factor of the DCCD-sensitive ATPase in different regions of the kidney.

Table II shows the effect of different inhibitors on Mg^{2+}-ATPase activity in various regions of the kidney. DCCD and filipin (36, 37) inhibited the ATPase activity by more than 60% in various regions. Vanadate, an inhibitor of gastric H^+-K^+-ATPase (38), failed to inhibit, whereas NEM inhibited ATPase by 15–20%. The results observed with NEM are in agreement with a recent study that failed to show inhibition of Mg^{2+}-ATPase activity in membranes from the renal medulla (5). We also measured the activity of this enzyme in the absence and presence of 10 μg/ml of oligomycin to ascertain that this ATPase activity was not secondary to mitochondrial contamination. This concentration of oligomycin inhibited mitochondrial ATPase activity by 95%. In contrast the DCCD-sensitive ATPase activity in various regions of the kidney was resistant to oligomycin, with minimal inhibition observed.

Table III shows the V_{max} and K_m for ATP and K_m for Cl^- for the DCCD-sensitive ATPase determined in various regions of the kidney. The V_{max} was variable, but the K_m for ATP was remarkably similar in the range of 54–68 μM in the various regions. The K_m for Cl^- was low in the range of 2–5 mM in various regions of the kidney. Figure 5 shows the ATPase activity measured in various regions of the kidney in the presence and absence of Cl^- (Cl^- was replaced by equimolar amounts of gluconate). It can be seen that the replace-

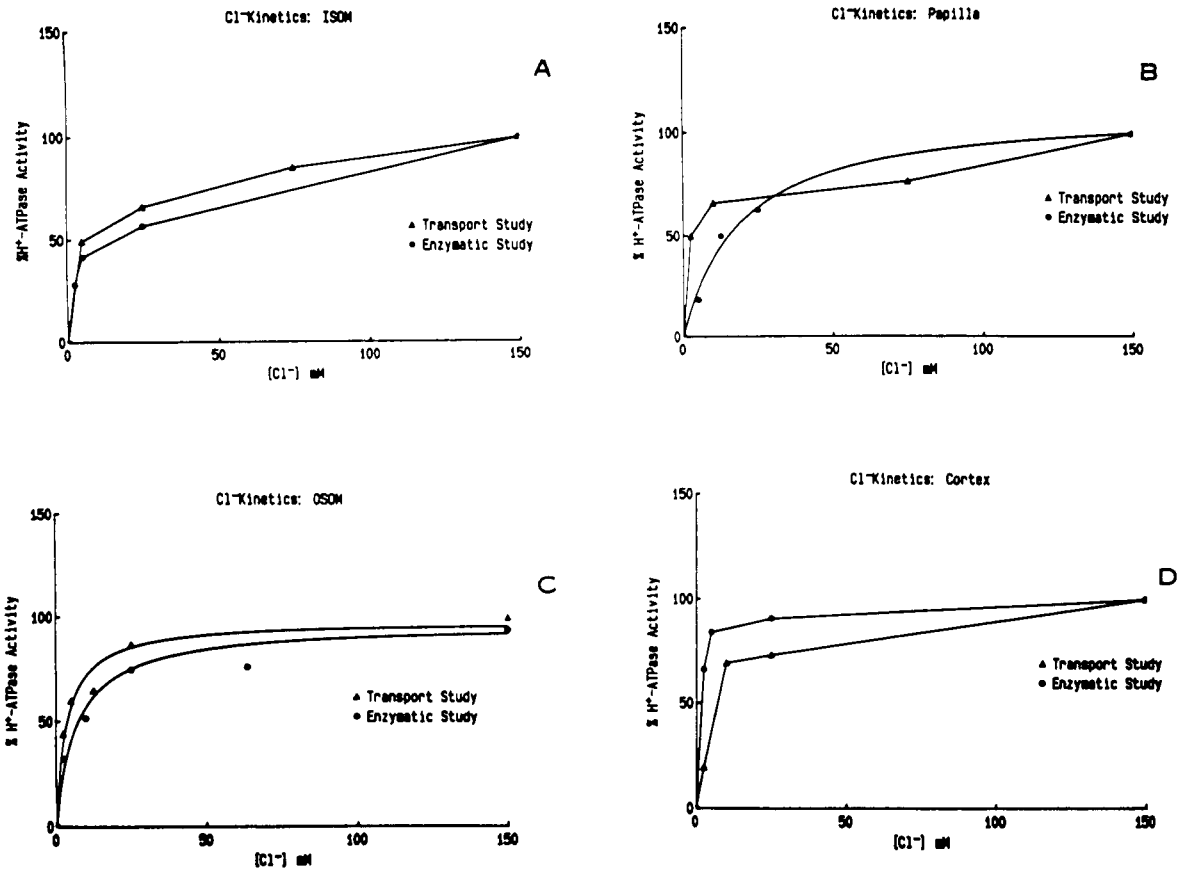

Figure 4. The effect of chloride on H^+-ATPase activity (DCCD-sensitive ATPase activity) and H^+ transport as assessed by acridine orange quenching on membranes vesicles from the (A) ISOM, (B) papilla, (C) OSOM, and (D) cortex.

Table II. Effect of Inhibitors on Mg^{2+}-ATPase Activity of Cortex, Outer and Inner Stripes of Outer Medulla, and Papilla[a]

Inhibitor	Percentage of inhibition			
	Cortex	OSOM	ISOM (15–25% fraction)	Papilla
DCCD (200 μM)	49 ± 3	67 ± 4	73 ± 5	81 ± 3
NEM (1 mM)	16 ± 3	15 ± 2	13 ± 3	21 ± 7
Filipin (70 μg)	27 ± 7	61 ± 4	70 ± 2	80 ± 2
Vanadate (100 μm)	—	2 ± 0.1	4 ± 0.2	0
Oligomycin[b] (10 μg/ml)	16 ± 0.8	16 ± 0.2	8 ± 1.0	15 ± 0.4

[a] ATPase activity was measured in the presence of ouabain (0.5 mM) and oligomycin (10 μg/ml), in the absence of calcium, and in the presence and absence of the inhibitor listed in the table.
[b] ATPase activity was measured as described in the Methods in the presence of ouabain (0.5 mM) and in the absence of calcium (n = 5 in each group). Values are means ± SE.

ment of chloride by gluconate decreased the ATPase activity by approximately 50% in all regions of the kidney. In a separate set of experiments, we determined the ATPase activity in the presence of varying concentrations of Cl^- (replaced by equimolar amounts of gluconate) to determine the K_m for Cl^-. To ascertain that the decrease in ATPase activity observed with gluconate was not secondary to the potential difference generated by the H^+ gradient in the absence of a permeant anion, we measured DCCD-sensitive ATPase in the absence and presence of the protonophore carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) (12). If the effect for gluconate to decrease ATPase activity was secondary to the H^+ gradient, then FCCP, by abolishing the H^+ gradient, should stimulate ATPase activity. In the experiments shown in Table IV, the effect of gluconate on decreasing DCCD-sensitive ATPase activity was of lesser magnitude than that seen in

Table III. V_{max} and K_m for ATP and K_m for Cl^- of DCCD-Sensitive Mg^{2+}-ATPase in Different Renal Regions[a]

Renal region	ATP		Cl
	V_{max}[b]	K_m (μM)	K_m (mM)
Cortex	0.11 ± 0.02	59.6 ± 9.5	4.7 ± 0.6
OSOM	0.78 ± 0.25	54.0 ± 6.8	2.3 ± 0.2
ISOM (15–25% fraction)	5.13 ± 1.18	56.6 ± 14.5	3.9 ± 0.9
Papilla	1.59 ± 0.25	68.0 ± 36.2	3.1 ± 0.9

[a] Values are means ± SE (n = 5 in each group).
[b] V_{max} is expressed as nmol/μg protein/min.

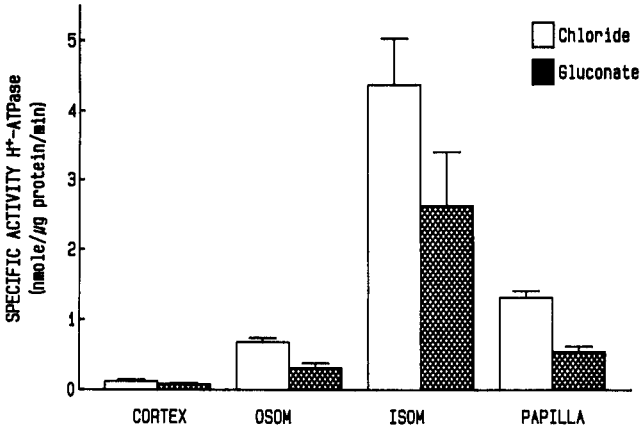

Figure 5. DCCD-sensitive ATPase activity in various regions of the kidney measured in the presence and in absence of chloride. Chloride was replaced by equimolar amounts of gluconate.

Table IV. Effect of FCCP on DCCD-Sensitive H^+-ATPase Activity (Percentage of Control)[a]

	Cl^- + FCCP	Gluconate	Gluconate + FCCP
Cortex	93.9 ± 4.6	66.9 ± 7.4	74.3 ± 7.8
OSOM	81.7 ± 5.7	76.5 ± 2.4	88.7 ± 5.9
ISOM (15–25% fraction)	91.5 ± 2.2	83.0 ± 8.8	80.6 ± 5.1
Papilla	92.7 ± 7.6	93.4 ± 4.2	80.8 ± 9.6

[a] Values are expressed as percentage of control measured in presence of chloride (n = 3–4 in each group).

Figure 5, probably because the membranes were leaky secondary to prolonged storage. Nevertheless, it is clear that the effect of gluconate is not reversed by the addition of FCCP. This indicates that effects of gluconate to decrease DCCD-sensitive ATPase activity is not due to generation of a H^+ gradient. These results are compatible with the interpretation that chloride directly affects the ATPase activity.

Discussion

Membrane Preparation and Measurement of DCCD-Sensitive ATPase Activity. The origin of the membranes used to characterize the ATP-dependent H^+ translocation in various regions of the kidney deserves discussion. The membrane vesicles prepared from the renal cortex were highly purified, as assessed by the enrichment in classical brush border enzyme markers, alkaline phosphatase, and γ-glutamyltransferase and the lack of enrichment in Na-K-ATPase. We believe that the membranes prepared from the OSOM originate mostly, if not exclusively, from the S_3 segment of the proximal tubule (29, 39). This contention is based on the fact that when tissue obtained for this region was examined by electron microscopy, it was observed that more than 90% of the tubules contained brush border membranes. In addition, the activity of γ-glutamyltransferase which was enriched in our membrane preparation, has been identified in the S_3 segment, but not in the cortical thick ascending limb, another structure present in the OSOM (40). Based on the above evidence, we assume that the majority of membranes prepared from the OSOM originated from the S_3 segment. The papillary membranes were a mixture of both luminal and basolateral membranes, as assessed by the enrichment in Na-K-ATPase activity, a marker for the basolateral membranes, and H^+-ATPase activity, which is thought to represent a marker for the luminal membranes.

The membranes prepared from the inner stripe of the outer medulla were divided into two fractions: The F_1 fraction was enriched 25-fold in DCCD-sensitive ATPase activity, with minimal enrichment in Na-K-ATPase, whereas the F_2 fraction was enriched 9-fold in Na-K-ATPase. If one assumes that the DCCD-sensitive ATPase represents, at least in part, H^+-ATPase, which is a marker of the luminal membrane of the collecting duct, then the F_1 fraction is predominantly luminal membrane, whereas the F_2 would represent a mixture of luminal and basolateral membranes. Since the thin limb and thick ascending limb contain H^+-ATPase, as assessed by immunocytochemistry techniques (28), the contribution of these structures to the enrichment of DCCD-sensitive ATPase activity in the F_1 fraction cannot be determined with certainty. It is clear, however, that the highest amount of DCCD-sensitive ATPase is present in medullary collecting ducts, as shown by studies performed in microdissected nephron segments and by immunocytochemistry techniques (1, 28). It is, therefore, reasonable to conclude that the F_1 fraction contains, at a minimum, luminal membranes of the medullary collecting duct.

DCCD-sensitive ATPase activity was measured in the presence of ouabain to inhibit Na-K-ATPase, in the absence of Ca^{2+} to inhibit Ca^{2+}-ATPase, in the presence

of oligomycin to inhibit mitochondrial ATPase, and in the absence and presence of DCCD. Under these conditions, DCCD-sensitive ATPase activity was disclosed in all regions of the kidney, with the highest activity in the medulla and the lowest in the cortex. The medullary and papillary vesicles are probably oriented inside out, as assessed by the fact that ATP addition to the outside promotes H^+ translocation, which suggests that the cytoplasmic side faces the outside. On the other hand, brush border membranes are right side out and, therefore, the ATP should not have access to the H^+-ATPase. Turrini *et al.* (10) have suggested that Mg^{2+}-dependent ATPase measured previously in the brush border membranes by other investigators represents (36, 37, 41, 42) an ATPase localized in the outside of the brush border membranes; thus, this activity does not represent the true H^+-ATPase activity, which is located in the inside (cytoplasmic side). They suggested that in order to measure the true H^+-ATPase activity, the membranes must be exposed to detergent to allow ATP entry into the vesicles.

In our studies, the membranes were permeabilized by raising the temperature of the medium, and this procedure has been successful in loading ATP into the vesicles. Additional evidence for the fact that ATP was able to enter the vesicles is the finding that the addition of detergent to our membrane vesicles did not enhance ATPase activity (data not shown). This finding suggests that the brush border membranes were permeable to ATP; therefore, our assay discloses the ATPase activity present in the cytoplasmic side of the vesicles.

Correlation between ATP-Dependent H^+ Translocation and DCCD-Sensitive ATPase Activity in Various Regions of the Kidney. Our studies clearly demonstrate that membranes from various regions of the kidney are capable of ATP-dependent H^+ translocation. This H^+ translocation was observed in the absence of Na^+, which indicates that it is not mediated by an Na-H antiporter. It is interesting to note that the inhibitors of vacuolar H^+-ATPase were capable of inhibition of H^+ translocation. Indeed, the inhibitors used showed variable degrees of inhibition of DCCD-sensitive ATPase activity, but completely inhibited H^+ translocation in response to ATP. Since DCCD and NEM inhibited both H^+ translocation and ATPase activity, it is reasonable to assume at least part of Mg^{2+}-ATPase activity (that which is inhibited by DCCD) represents H^+-ATPase activity. Under the assay condition used in the present study, we measured Mg^{2+}-dependent ATPase activity in various regions of the kidney. This ATPase was resistant to oligomycin and vanadate, which suggests that it does not represent mitochondrial contamination, and this ATPase is different from that found in the stomach (38). This Mg^{2+}-ATPase was sensitive to DCCD and filipin and was only mildly sensitive to NEM. Although NEM has been used to characterize the Mg^{2+}-ATPase in various studies, the pattern of inhibition by NEM is of variable degree. Some investigators have found no inhibition of ATPase in the medulla (5), whereas other investigators have found only 10% inhibition in the medulla and in the cortex (1). Obviously, the degree of inhibition elicited by NEM is variable, and it is clear, therefore, that the degree of inhibition cannot be used to characterize this ATPase. Immunocytochemistry techniques utilizing monoclonal antibody have identified H^+-ATPase in various segments of the kidney in variable amounts, as assessed by the degree of labeling of the segments (28). These studies suggest that the H^+-ATPase in the various nephron segments is basically the same. The activity of Mg^{2+}-ATPase measured in the present study was highest in the medulla and lowest in the cortex, with intermediate values in the papilla followed by the OSOM. The K_m for ATP, however, was very close among the different regions and it was of high affinity. These results are, therefore, in accord with the suggestion that this Mg^{2+}-ATPase is essentially the same in the different renal regions.

The ATPase activity and ATP-dependent H^+ translocation exhibit variable degrees of sensitivity to anion composition of the medulla. In some tissues, the H^+ translocation is critically chloride dependent, whereas in others, it is chloride independent, and, in others still, H^+ transport is only partially dependent upon chloride (13, 14, 18, 23). The renal DCCD-sensitive ATPase and H^+ translocation were critically dependent upon chloride in that removal of chloride reduced transport and enzymatic activities by approximately 50%. The K_m for chloride for both transport and enzymatic activities was very low in the range of 2–5 m*M*. This effect of chloride on stimulation of transport and enzymatic activities could be mediated either by the presence of chloride channel and/or by a catalytic site for chloride on the ATPase. The finding that the chloride channel inhibitor diphenylamine-2 carboxylic acid blocked H^+ translocation clearly suggests that a chloride channel is present in these membranes. Similar findings have been described for the H^+-ATPase of corn root membrane vesicles (12), and, overall, these findings are consistent with a direct effect of chloride in activating the H^+-ATPase. In addition, chloride permeates through the channel and dissipates the unfavorable potential difference generated by H^+ transport and thereby enhances H^+ transport. Bennett and Spanswick (12) found that the K_m for chloride to stimulate ATPase activity and H^+ transport were very similar and, on this basis, they suggested that the H^+-ATPase and the chloride channel are closely associated. The results of the present study are compatible with the suggestion of Bennett and Spanswick (12). Of interest is the fact that in the presence of chloride, FCCP failed to stimulate ATPase activity. This can be explained by the fact that, in the

presence of chloride, an appreciable H^+ gradient is not generated, or alternatively, under the assay conditions, the vesicles are leaky, thus preventing the generation of a sizable H^+ gradient.

The finding that hydrogen ion transport is particularly dependent upon chloride may have relevance to normal renal physiology. It is well known that hydrogen ion secretion in the distal nephron is sensitive to voltage in that a positive voltage inhibits hydrogen ion secretion whereas a negative voltage stimulates hydrogen ion secretion. The presence of an electrogenic H^+-ATPase in the lumen of distal nephron would generate a positive luminal potential difference, and this adverse electrochemical gradient would slow down the proton pump. The presence of a chloride channel that would be stimulated by hydrogen ion secretion would "shunt" the voltage generated by the pump and would allow hydrogen ion secretion to continue.

In summary, our data show a DCCD-sensitive ATPase activity in various regions of the kidney, with similar K_m for ATP but variable V_{max}. This Mg^{2+}-ATPase is capable of H^+ translocation, and both the enzymatic and H^+ transport activities are critically dependent upon chloride, which suggests that chloride enhances ATPase activity and H^+ translocation but acts at a channel that is closely associated with a catalytic site of the enzyme.

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