Communication

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 271, No. 13, Issue of March 29, pp. 7277–7280, 1996

The Rat Distal Colon P-ATPase α Subunit Encodes a Ouabain-sensitive H^+,K^+-ATPase*

(Received for publication, January 19, 1996)

M. Cougnon‡§, G. Planelles‡, M. S. Crowson¶, G. E. Shull¶, B. C. Rossier‖, and F. Jaisser‡‡**

*From ‡INSERM, U323, Faculté de Médecine Necker, Université Paris V, F-75015 Paris, France, the ¶University of Cincinnati College of Medicine, Cincinnati, Ohio 45267, the ‖Institut de Pharmacologie et Toxicologie, Rue du Bugnon 27, CH-1005 Lausanne, Switzerland, and **Institut Curie, UMR144, 12 rue Lhomond, F-75005 Paris, France*

The functional properties and the pharmacological profile of the recently cloned cDNA colonic P-ATPase α subunit (Crowson, M. S., and Shull, G. E. (1992) *J. Biol. Chem.* 267, 13740–13748) were investigated by using the *Xenopus* oocyte expression system. *Xenopus* oocytes were injected with α subunit cRNAs from *Bufo marinus* bladder or rat distal colon and/or with β subunit cRNA from *B. marinus* bladder. Two days after injection, K^+ uptake was measured by using $^{86}Rb^+$ as a K^+ surrogate, and pH measurements were performed by means of ion-selective microelectrodes. Co-injection of α and β subunit cRNAs lead to a large increase in $^{86}Rb^+$ uptake, an intracellular alkalinization, and an extracellular medium acidification, as compared to α or β injection alone. These results indicate that the colonic P-ATPase α subunit, like the bladder α subunit, acts as a functional H^+,K^+-ATPase, and that co-expression of α and β subunits is required for the function. External K^+ activation of the $^{86}Rb^+$ uptake had a $K_{1/2}$ ~ 440 μM for the bladder isoform (consistent with the previously reported value (Jaisser, F., Horisberger, J. D., Geering, K., and Rossier, B. C. (1933) *J. Cell Biol.* 123, 1421–1431)) and a $K_{1/2}$ ~ 730 μM for the colonic isoform. Sch28080 was ineffective to reduce $^{86}Rb^+$ uptake whereas ouabain inhibited the activity expressed from rat colon α subunit with a K_i of 970 μM when measured at the V_{max} of the enzyme. We conclude that, when expressed in *Xenopus* oocytes, the rat colon P-ATPase α subunit encodes a ouabain-sensitive H^+,K^+-ATPase.

Both colon and kidney are involved in chronic adaptation to K^+ homeostasis (1, 2). Specific transmembrane proteins in colonic and renal cells are responsible for K^+ transport: whereas K^+ secretion is mediated by diffusion through potassium channels, apical K^+ absorption occurs against an adverse electrochemical transmembrane gradient, *i.e.* via active process(es) (1). Primary active K^+ transport systems, requiring ATP hydrolysis, have been described both in colon and kidney by physiological and biochemical techniques (3–7). However, the molecules responsible for these colonic and renal K^+-ATPase activities are not yet defined.

Expression of gastric H^+,K^+-ATPase α and β subunit cRNAs has been reported recently all along the renal collecting duct (8, 9), but not in distal colon (10). The gastric H^+,K^+-ATPase may therefore participate in K^+ handling in the kidney, and may account for the ouabain-resistant, Sch28080-sensitive K^+ reabsorption process that has been described in this tissue (3, 11, 12). Recently, a novel P-type ATPase α subunit cDNA has been cloned from a rat distal colon cDNA library (10, 13). It clearly belongs to a novel subgroup of the Na^+,K^+/H^+,K^+-ATPases gene family and is equally related to, but distinct from, either the Na^+,K^+-ATPase and the gastric H^+,K^+-ATPase. In colon, the expression of this P-ATPase is restricted to the most superficial cells of the distal colon (13, 14). In the kidney, it is not expressed in rat maintained on a standard diet, but acute and chronic K^+ deprivation markedly stimulates its mRNA expression in the outer medullary collecting duct, but not in other nephron segments (14). Primary structure analysis suggests that this novel P-ATPase α subunit may encode a K^+- or H^+,K^+-ATPase (10, 13). Its putative function, its restricted tissue- and cell-specific expression, and its physiological regulation strongly suggests that it corresponds to a K^+(H^+,K^+)-ATPase involved in colon and renal K^+ reabsorption.

To test this hypothesis, we have analyzed the properties of the colonic P-ATPase α subunit using a functional assay previously developed in the *Xenopus laevis* oocyte for the expression of the toad bladder H^+,K^+-ATPase (15). We demonstrate that this P-ATPase is indeed a ouabain-sensitive H^+,K^+-ATPase, which may therefore be involved in the ouabain-sensitive K^+ absorption reported in both distal colon and the distal nephron.

MATERIALS AND METHODS

cRNA Synthesis and Expression in Xenopus Oocytes—The 3.9-kilobase full-length cDNA encoding the putative colonic H^+,K^+-ATPase α subunit (α_c) was reconstructed from two previously described overlapping cDNAs (10) and subcloned into the pSD3 vector containing a 110-base pair poly(A)$^+$ tail before the *Hin*dIII linearization site (16). In order to increase translatability, the natural 5′-untranslated region was replaced by that of the *Bufo marinus* β_1 subunit, as described previously for the *Xenopus* and murine gastric H^+,K^+-ATPase α subunit (17). The *B. marinus* bladder α subunit (α_{bl}) and β subunit (β_{bl}) cRNAs were obtained as described (15). *Xenopus* oocytes were injected with either 10 ng of α subunit or 2 ng of β subunit cRNAs, alone or in combination.

Rubidium Uptake—Two days after cRNA injection of the oocytes, $^{86}Rb^+$ uptake measurements were performed. Batches of 8–10 oocytes were preincubated at room temperature for 10 min in a K^+-free assay solution containing (in mM) NaCl, 90; $MgCl_2$, 1; $Ca(NO_3)_2$, 0.41; $BaCl_2$, 5 (buffered at pH 7.4 with Tris/NaOH) supplemented with 10 μM ouabain to inhibit the endogenous Na^+,K^+-ATPase, as described previously (15). Immediately after, oocytes were incubated in an assay solution containing 5 μCi/ml $^{86}Rb^+$, at various K^+ and inhibitors concentrations in order to determine activation ($K_{1/2}$) and inhibition (K_i) constants. Individual oocytes were lysed in the presence of 100 μl of 5% sodium dodecyl sulfate and mixed with 2 ml of scintillation medium for counting.

pH Measurements—Intra- and extracellular pH measurements were performed by using double-barrelled (pH-selective and conventional) microelectrodes. Procedures for manufacturing these microelectrodes were as described elsewhere (18). In this study, the liquid H^+ ionophore 95297 from Fluka was used. Before use, microelectrodes were bevelled

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Supported by a fellowship from the Société Française de Néphrologie.

‡‡ Supported by an EMBO long term fellowship. To whom correspondence should be addressed. Tel.: 33-1-4234-6362; Fax: 33-1-4234-6377.

TABLE I

Expression in X. laevis oocytes of cRNAs encoding colonic (c) or toad bladder (bl) P-ATPase α and β subunits: effects on $^{86}Rb^+$ uptake, intracellular pH (pH_i) and extracellular pH (pH_o)

Results expressed as means ± S.E.; n = number of oocytes.

	β_{bl}	$\alpha_c\ \beta_{bl}$	$\alpha_{bl}\ \beta_{bl}$
$^{86}Rb^+$ uptake (pmol/min/oocyte)	7 ± 1 (n = 23)	60 ± 2.5[a] (n = 37)	33 ± 1.5[a] (n = 42)
pH_i	7.36 ± 0.03 (n = 9)	7.85 ± 0.05[b] (n = 16)	7.91 ± 0.03[b] (n = 10)
pH_o	7.75 ± 0.02 (n = 5)	5.8 ± 0.1[a] (n = 9)	

[a] Level of significance ($p < 0.001$) assessed by comparison of the results to the first column.

[b] Level of significance ($p < 0.01$) assessed by comparison of the results to the first column.

on a microgrinder (De Marco Engineering, Switzerland). For a 10-fold decrease in proton activity (pH of the testing solutions 6.4–7.4 for intracellular measurements and 6.8–7.8 for extracellular measurements), the slope S of the microelectrodes was $50 < S < 57$ mV.

Intracellular pH (pH_i) was calculated by the relationship: $pH_i = pH_{ref} - (V_H - V_m)/S$, where pH_{ref} is the reference solution pH, V_H the measured electrochemical potential difference for H^+, V_m the transmembrane potential difference, S the slope of the microelectrodes.

Extracellular pH measurements were performed at room temperature on individual oocytes incubated in a 1-μl droplet (surrounded by oil) of a weakly buffered solution, as described previously (15). In some experiments, 1 mM ouabain was added to the external solution. The double-barrelled selective microelectrode was introduced into the droplet in order to measure the pH change of the solution. The conventional barrel was used as reference. Extracellular pH values reported here were obtained after 20 min of incubation.

Statistics—Results are expressed as means ± S.E. Statistical significance of the results was assessed using the unpaired Student's t test.

RESULTS AND DISCUSSION

Co-expression in *Xenopus laevis* oocytes of synthetic cRNAs encoding the colonic P-ATPase α subunit (10) and the toad urinary bladder β subunit (19) leads to a large increase in the uptake of $^{86}Rb^+$, a surrogate of K^+ used as a tracer, when compared to oocytes expressing the bladder β subunit alone as shown in Table I. $^{86}Rb^+$ uptake per oocyte is about twice that measured under the same experimental conditions in *Xenopus* oocytes co-expressing the toad bladder α and β subunits (Table I). $^{86}Rb^+$ uptake in *Xenopus* oocytes injected with the colonic P-ATPase α subunit alone is not different from those of oocytes injected with water (data not shown). Thus, co-expression of the colonic α subunit together with a β subunit is required for functional expression, as previously reported for the Na^+,K^+-ATPase (20), the gastric H^+,K^+-ATPase (17), and the toad bladder H^+,K^+-ATPase (15).

Measurement of the intracellular pH of injected oocytes shows that the co-expression of the colonic P-ATPase α subunit with the toad bladder β subunit induces an intracellular alkalinization, when compared to oocytes expressing the β_{bl} subunit alone; similar findings were observed in oocytes expressing the bladder H^+,K^+-ATPase (see Table I), as described previously (15). On the other hand, a significant pH decrease in an extracellular droplet surrounding the co-injected oocytes is observed, whereas in the same conditions, extracellular pH is not modified in the presence of oocytes expressing the β_{bl} subunit alone (Table I). Taken together, these results indicate that, when co-expressed in *Xenopus* oocytes with the β_{bl} subunit, the colonic P-ATPase α subunit acts as a functional H^+,K^+-ATPase.

We next evaluated the activation of the colonic H^+,K^+-ATPase by external K^+. Measurement of $^{86}Rb^+$ uptake was performed using increasing concentrations of extracellular K^+, in oocytes expressing either the rat colonic H^+,K^+-ATPase or the toad urinary bladder H^+,K^+-ATPase α subunits, with the same β subunit, *i.e.* the bladder β subunit. $K_{1/2}$ for K^+ was estimated to be ≈730 μM for the colonic H^+,K^+-ATPase (Fig. 1); a $K_{1/2} \approx 440$ μM for K^+ was found for the bladder H^+,K^+-ATPase, a value similar to that previously reported (15). The $K_{1/2}$ for K^+ of the colonic isoform is close to what has been measured for the K^+-activated ATPase activities in an apical membrane preparation of rat distal colon by Del Castillo *et al.* (6) (500–750 μM) and to the K_m of K^+ absorptive fluxes measured in the distal colon (520 μM) (7). Interestingly, the Hill coefficient determined for K^+ activation of the colonic and bladder H^+,K^+-ATPases is different (Hill coefficient: $\alpha_c\beta_{bl}$, 0.98 ± 0.067, n = 4; $\alpha_{bl}\beta_{bl}$, 2.25 ± 0.39, n = 3; $p < 0.01$). A Hill coefficient of 2 for the bladder H^+,K^+-ATPase strongly suggests the presence of two or more binding sites with strong cooperativity. Although a Hill coefficient of one, as observed for the rat colonic H^+,K^+-ATPase, does not exclude the presence of multiple binding sites, the very clear difference in Hill coefficient between the two types of H^+,K^+-pump suggests a difference of stoichiometry, or at least a significant difference in the interaction between binding sites. A stoichiometry of 2 K^+/2 H^+/1 ATP has been proposed for the gastric H^+,K^+-pump (21), but, at present, nothing is known about this characteristic in the other H^+,K^+ pumps.

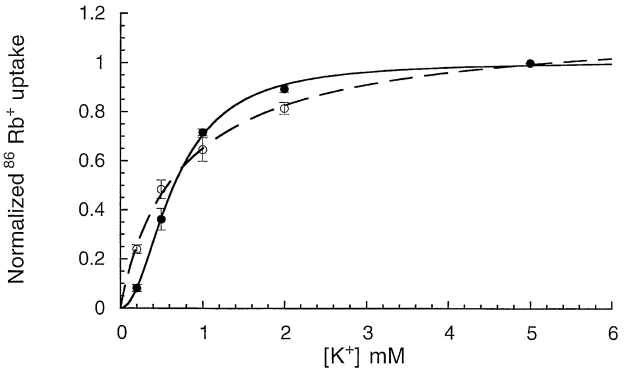

FIG. 1. **K^+ dose-dependent activation of $^{86}Rb^+$ uptake.** Results are normalized at 5 mM K^+ and expressed as mean ± S.E.; n = 24–40, from 3–4 independent experiments, depending on the K^+ concentration used. Curve-fitting is to a single model. ○, rat distal colon $\alpha_{H,K}$/toad bladder $\beta_{H,K}$; ●, toad bladder $\alpha_{H,K}$/toad bladder $\beta_{H,K}$.

Functional expression of the rat colonic H^+,K^+-ATPase allows us to analyze its pharmacological profile. In the rat distal colon, K^+ absorption appears to be mediated by two different apical Na^+-independent K^+-activated ATPases (5, 6). Their pharmacological characteristics differ from those of both the Na^+,K^+-ATPase and the gastric H^+,K^+-ATPase. One colonic K^+-activated ATPase is sensitive to vanadate and to ouabain but not to *N*-ethylmaleimide (inhibitor of V-type H^+-ATPase) or to omeprazole and Sch28080 (inhibitor of the gastric H^+,K^+-ATPase), while the other one is ouabain-insensitive but partly Sch28080-sensitive (5, 6). The rat distal colon H^+,K^+-ATPase expressed in *Xenopus* oocytes is not sensitive to 500 μM Sch28080 (% inhibition: 2.5 ± 9.5, n = 5), as compared to the moderately sensitive bladder H^+,K^+-ATPase (% inhibition: 41.6 ± 3.4, n = 6). We next examined the effect of ouabain on the rat colon H^+,K^+-ATPase activity. Fig. 2 shows that the colonic H^+,K^+-ATPase studied in this paper is sensitive to ouabain, with a K_i of 970 μM, when the assay is performed at V_{max}, in the presence of 5 mM K^+. Extracellular acidification is also blocked by 1 mM ouabain (pH unit, 7.7 ± 0.1, n = 3, in the presence of ouabain, as compared to 5.8 ± 0.1, n = 9, in its absence). Since it is known that K^+ has a competitive effect on ouabain inhibition of the Na^+,K^+-ATPase (22) and the toad bladder H^+,K^+-ATPase (15), we evaluated the effect of extracellular K^+ concentration on ouabain inhibition of $^{86}Rb^+$ uptake. Extracellular K^+ concentration affects ouabain affinity

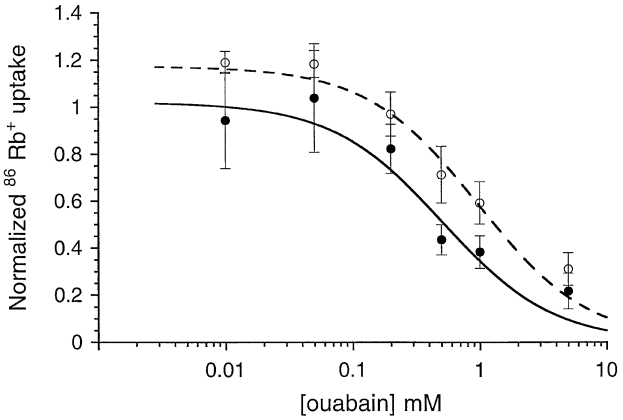

FIG. 2. **Dose-dependent inhibition of $^{86}Rb^+$ uptake by ouabain.** Results are normalized to results obtained in the absence of ouabain and are expressed as mean ± S.E.; n = 16–40, from 2–5 independent experiments, depending on the ouabain concentration used. ○, rat distal colon $\alpha_{H,K}$/toad bladder $\beta_{H,K}$; ●, toad bladder $\alpha_{H,K}$/toad bladder $\beta_{H,K}$.

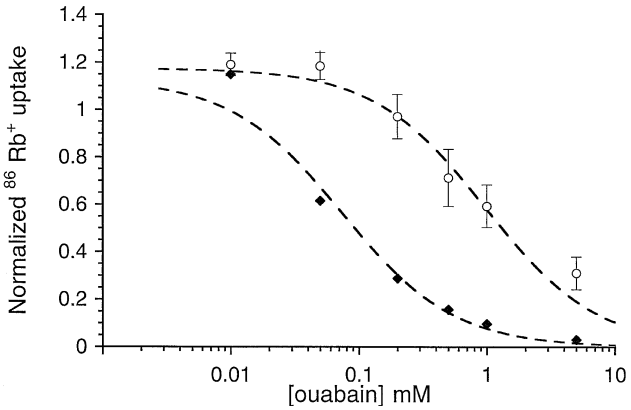

FIG. 3. **Dose-dependent inhibition of $^{86}Rb^+$ uptake by ouabain at two different concentrations of extracellular K^+, 0.2 mM (♦) and 5 mM (○), in oocytes injected with rat distal colon $\alpha_{H,K}$/toad bladder $\beta_{H,K}$ cRNAs.** Results are normalized in the absence of inhibitor and are expressed as mean ± S.E. One experiment was performed for the dose-dependent inhibition of $^{86}Rb^+$ uptake performed in the presence of 0.2 mM K^+ using 8–10 oocytes per K^+ concentration. Data for the dose-dependent inhibition of $^{86}Rb^+$ uptake performed in the presence of 0.55 mM K^+ are those reported in Fig. 2.

since K_i measured in the presence of 0.2 mM K^+ is about 70 μM, as compared to 970 μM in the presence of 5 mM K^+ (Fig. 3). It is concluded from the above experiments that the colonic H^+,K^+-ATPase presently expressed in *Xenopus* oocytes is ouabain-sensitive and Sch28080-insensitive.

The inhibition constant for ouabain measured in the presence of 5 mM K^+ differs from the one reported in the literature for the distal colon ouabain-sensitive K^+-ATPase by Del Castillo *et al.* (6). In that paper, an inhibition constant of 100 μM was reported when K^+-activated ATPase activity was measured in the presence of 20 mM KCl. As demonstrated above, the ouabain affinity clearly depends on the extracellular K^+ concentration. Thus, the ouabain-sensitive K^+-ATPase measured by Del Castillo *et al.* (6) would be even more sensitive to ouabain if the K^+ concentration was 5 mM instead of 20 mM. Several explanations for this discrepancy can be proposed. First, different methodological procedures were used; however, we have previously demonstrated that the determination of ouabain inhibition kinetics in *Xenopus* oocyte is comparable to results obtained with other techniques: the ouabain K_i of *B. marinus* Na^+,K^+-ATPase in a purified enzyme preparation from the bladder (23), in the TBM cell line (20), or in oocytes expressing the *B. marinus* $\alpha_1\beta_1$ Na^+,K^+-ATPase subunits (20) was determined to be 100, 56, and 40 μM, respectively.

Second, one should consider that the β subunit physiologically associated with the colonic α subunit may confer a different pharmacological profile to the pump. The β subunit associated *in vivo* with the colonic α subunit is not yet defined. The gastric H^+,K^+-ATPase β subunit is not expressed in the colon (24), while the Na^+,K^+-ATPase β_1 subunit is (25). Marxer *et al.* (25) reported that a protein sharing common epitopes with the Na^+,K^+-ATPase β_1 subunit was indeed present in the apical membrane of the rat distal colon (25). Whether this subunit corresponds to the Na^+,K^+-ATPase β_1 or to an unidentified β subunit isoform remains unknown. It has been well established that the β subunit affects the functional properties of the Na^+,K^+-ATPase (26, 27) and, to some extent, those of the gastric H^+,K^+-ATPase (28, 29). To date, the ouabain sensitivity of the diverse αβ heterodimers already tested in *Xenopus* oocytes or in the insect Sf-9 cells was not significantly affected by the type of β isoform used in the assay (20, 30). One exception is the rat gastric β subunit which slightly affects the K_d for ouabain binding from 10.1 to 5.8 nM, when associated with the sheep Na^+,K^+-ATPase α_1 subunit (27).

A third possible explanation for the discrepancy between the results of the current study and the previously reported ouabain affinity of the colonic ouabain-sensitive K^+-ATPase (6) would be that we are not dealing with the same K^+-ATPase. In fact, the so-called ouabain-resistant K^+-ATPase found in distal colon may not be fully resistant to ouabain. The ouabain-resistant fraction of the colonic K^+-activated ATPase activities was determined as the K^+-ATPase activity resistant to 5 mM ouabain in the presence of 20 mM external K^+ (6) or to 1 mM ouabain in the presence of 15 mM external K^+ (5). This remaining activity could either correspond to a fully resistant K^+-ATPase or to the residual fraction of a moderately sensitive K^+-ATPase, as described here. Indeed, as shown in Fig. 2, inhibition with 1 mM ouabain of the colonic H^+,K^+-ATPase expressed in oocytes resulted in only 50% inhibition when measured in the presence of 5 mM external K^+.

Interestingly, the pharmacological profile described here, *i.e.* ouabain sensitivity and Sch28080 insensitivity, is opposite to the one reported for the expression of the same α subunit cDNA in insect cells (31). These authors report on functional expression of the colonic P-ATPase α subunit after infection of Sf9 cells with a recombinant baculovirus. Sf9 infection induces a 3-fold increase in K^+-ATPase activity, measured as a Na^+- and Mg^{2+}-independent K^+-activated ATPase activity (31). It should be noted that the K^+-ATPase activity was measured after expression of the colonic α subunit alone, without β subunit. These data contrast with what has been reported for the Na^+,K^+-ATPase (32, 33) or the gastric H^+,K^+-ATPase (34) in the same functional expression system. In these cases, coexpression of α and β subunits was an absolute prerequisite for the induction of a Na^+,K^+- or H^+,K^+-ATPase activity (32–34). Thus, the K^+-activated ATPase activity reported after expression of the colonic α subunit alone is difficult to reconcile with previous studies. This ATPase activity may reflect the functional expression of colonic α subunit oligomers, as reported recently by Blanco *et al.* (35) for Na^+,K^+-ATPase α/α oligomers, or heterologous heterodimers formed with insect proteins, like insect Na^+,K^+-ATPase α and/or β subunits. Such complexes may have specific functional and pharmacological properties which may explain the differences with the pharmacological characteristics reported here.

The colonic H^+,K^+-ATPase α subunit has been reported recently to be expressed within the kidney when rats were subjected to acute or chronic K^+ deprivation. Expression was

strictly restricted to the outer medullary collecting duct cells and was not affected by aldosterone (14). The role of the "colonic" H^+,K^+-ATPase in renal K^+ handling and renal K^+ balance remains to be defined. Rat distal nephron micropuncture studies (36, 37), as well as studies performed on isolated perfused rat kidneys (38), have shown that renal K^+ absorption is inhibited by ouabain, suggesting that "ouabain acts directly on distal potassium transport by inhibiting active uptake of this ion at the luminal cell membrane" (36). The molecular basis underlying this active K^+ transport has not been determined. Hayashi and Katz (39) reported the presence of a ouabain-sensitive K^+-ATPase in the outer medullary collecting duct which may explain the effect of ouabain on renal K^+ absorption. Altogether, the physiological data from the literature, the functional results reported here, as well as the cell-specific expression of the colonic H^+,K^+-ATPase in the kidney, strongly suggests that the "colonic" ouabain-sensitive H^+,K^+-ATPase is involved in renal K^+ handling during K^+ deprivation. In rats maintained on a standard diet, a ouabain-resistant, Sch28080-sensitive K^+ transport has been reported in the cortical and medullary collecting ducts (3). This K^+ transport is probably mediated by a ouabain-resistant, Sch28080-sensitive K^+-ATPase present in the same nephron segments (12) and may correspond to the gastric H^+,K^+-ATPase α subunit reported to be expressed all along the distal nephron in rats on a standard diet (8). Whether this ouabain-resistant K^+ reabsorption/K^+-ATPase is indeed important for renal K^+ conservation remains unclear. Dissection of the specific role of the ouabain-sensitive and the ouabain-resistant K^+(H^+,K^+)-ATPases in renal K^+ handling will require further experiments. Genetically modified animals, such as knock-out mice having a null mutation in the colonic H^+,K^+-ATPase α subunit gene and/or the gastric H^+,K^+-ATPase α subunit gene, may be useful to specifically address this question.

Acknowledgment—We thank Jean-Daniel Horisberger for critical reading and suggestions.

REFERENCES

1. Wright, F. S., and Giebisch, G. (1992) in *The Kidney. Physiology and Pathophysiology* (Seldin, D. W., and Giebisch, G., eds) pp. 2209–2248, Raven Press, New York
2. Agarwal, R., Afzalpurkar, R., and Fordtran, J. S. (1994) *Gastroenterology* **107,** 548–571
3. Cheval, L., Barlet-Bas, C., Khadouri, E., Feraille, E., Marsy, S., and Doucet, A. (1991) *Am. J. Physiol.* **260,** F800–F805
4. Gifford, D., Rome, L., and Galla, J. H. (1992) *Am. J. Physiol.* **262**, F692–F695
5. Tabuchi, Y., Takeguchi, M., Asano, S., and Takeguchi, N. (1992) *Jpn. J. Physiol.* **42,** 577–589
6. Del Castillo, J. R., Rajendran, V. M., and Binder, H. J. (1991) *Am. J. Physiol.* **261,** G1005–G1011
7. Sweiry, J. H., and Binder, H. J. (1990) *J. Physiol. (Lond.)* **423,** 155–170
8. Kraut, J. A., Starr, F., Sachs, G., and Reuben, M. (1995) *Am. J. Physiol.* **268,** F581–F587
9. Callaghan, J. M., Tan, S. S., Khan, M. A., Curran, K. A., Campbell, W. G., Smolka, A. J., Toh, B.-H., Gleeson, P. A., Wingo, C. S., Cain, B. D., and Van Driel, I. R. (1995) *Am. J. Physiol.* **268,** F363–F374
10. Crowson, M. S., and Shull, G. E. (1992) *J. Biol. Chem.* **267,** 13740–13748
11. Zhou, X., and Wingo, C. S. (1992) *Am. J. Physiol.* **263,** F43–F48
12. Doucet, A., and Marsy, S. (1987) *Am. J. Physiol.* **253,** F418–F423
13. Jaisser, F., Coutry, N., Farman, N., Binder, H. J., and Rossier, B. C. (1993) *Am. J. Physiol.* **265,** C1080–C1089
14. Jaisser, F., Escoubet, B., Coutry, N., Eugene, E., Bonvalet, J. P., and Farman, N. (1996) *Am. J. Physiol.*, in press
15. Jaisser, F., Horisberger, J. D., Geering, K., and Rossier, B. C. (1993) *J. Cell Biol.* **123,** 1421–1431
16. Horisberger, J. D., Jaunin, P., Good, P. J., Rossier, B. C., and Geering, K. (1991) *Proc. Natl. Acad. Sci U. S. A.* **88,** 8397–8400
17. Matthews, P. M., Klaeys, D., Jaisser, F., Geering, K., Horisberger, J. D., Kraehenbühl, J. P., and Rossier, B. C. (1995) *Am. J. Physiol.* **268,** C1207–C1214
18. Anagnostopoulos, T., and Planelles, G. (1987) *J. Physiol. (Lond.)* **393,** 73–89
19. Jaisser, F., Horisberger, J. D., and Rossier, B. C. (1993) *Pfluegers Arch. Eur. J. Physiol.* **425,** 446–452
20. Jaisser, F., Canessa, C. M., Horisberger, J. D., and Rossier, B. C. (1992) *J. Biol. Chem.* **267,** 16895–16903
21. Rabon, E. C., and Reuben, M. A. (1990) *Annu. Rev. Physiol.* **52,** 321–344
22. Forbush, B. I. I. I. (1983) *Curr. Top. Membr. Transp.* **19,** 167–201
23. Cortas, N., and Walzer, M. (1971) *Biochim. Biophys. Acta* **249,** 181–187
24. Reuben, M. A., Lasater, L. S., and Sachs, G. (1990) *Proc Natl. Acad. Sci. U. S. A.* **87,** 6767–6771
25. Marxer, A., Stieger, B., Quaroni, A., Kashgarian, M., and Hauri, H. P. (1989) *J. Cell Biol.* **109,** 1057–1069
26. Jaisser, F., Jaunin, P., Geering, K., Rossier, B. C., and Horisberger, J.-D. (1994) *J. Gen. Physiol.* **103,** 605–623
27. Eakle, K. A., Seong kim, K., Kabalin, M. A., and Farley, R. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 2834–2838
28. Chow, D. C., Browning, C. M., and Forte, J. G. (1992) *Am. J. Physiol.* **263,** C39–C46
29. Chow, D. C., and Forte, J. G. (1995) *Am. J. Physiol.* **265,** C1562–C1570
30. Blanco, G., Koster, J., Sanchez, G., and Mercer, R. W. (1995) *Biochemistry* **34,** 319–325
31. Lee, J., Rajendran, V. M., Mann, A. S., Kashgarian, M., and Binder, H. J. (1995) *J. Clin. Invest.* **96,** 2002–2008
32. De Tomaso, A. W., Blanco, G., and Mercer, R. W. (1993) *J. Cell Biol.* **127,** 55–69
33. Blanco, G., Jian Xie, J., and Mercer, R. W. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 1824–1828
34. Klaasen, C. H. W., Van Uem, T. J. F., De Moel, M. P., De Caluwe, G. L. J., Swarts, H. G. P., and De Pont, J. J. H. H. M. (1993) *FEBS Lett.* **329,** 277–282
35. Blanco, G., De Tomaso, A. W., Koster, J., Xie, Z. J., and Mercer, R. W. (1994) *J. Biol. Chem.* **269,** 23420–23425
36. Duarte, C. G., Chomety, F., and Giebisch, G. (1971) *Am. J. Physiol.* **221,** 632–639
37. Strieder, N., Khuri, R., Wiederholt, M., and Giebisch, G. (1974) *Pfluegers Arch. Eur. J. Physiol.* **349,** 91–107
38. Hayashi, M., and Katz, A. I. (1987) *Am. J. Physiol.* **252,** F447–F452
39. Hayashi, M., and Katz, A. I. (1987) *Am. J. Physiol.* **252,** F437–F446