Distinct Regulatory Effects of the Na,K-ATPase γ Subunit*

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The two variants of the γ subunit of the rat renal sodium pump, γa and γb, have similar effects on the Na,K-ATPase. Both increase the affinity for ATP due to a shift in the enzyme’s E1 ↔ E2 conformational equilibrium toward E1. In addition, both increase K+ antagonism of cytoplasmic Na+ activation. To gain insight into the structural basis for these distinct effects, extramembranous N-terminal and C-terminal mutants of γ were expressed in rat α1-transfected HeLa cells. At the N terminus, the variant-distinct region was deleted (γNΔ7) or replaced by alanine residues (γN7A). At the C terminus, four (γCΔ4) or ten (γCΔ10) residues were deleted. None of these mutations abrogates the K+/Na+ antagonism as evidenced in a similar increase in K+Na+ observed at high (100 mm) K+ concentration. In contrast, the C-terminal as well as N-terminal deletions (γNΔ7, γCΔ4, and γCΔ10) abolished the decrease in K+ATP seen with wild-type γa or γb. It is concluded that different regions of the γ chain mediate the distinct functional effects of γa and the effects can be long-range. In the transmembrane region, the impact of G41R replacement was analyzed since this mutation is associated with autosomal dominant renal Mg2+-wasting in man (Meij, I. C., Koenderink, J. B., van Bokhoven, H., Assink, K. F. H., Groenestege, W. T., de Pont, J. J. H. M., Bindels, R. J. M., Monnens, L. A. H., Van den Heuvel, L. P. W. J., and Knoers, N. V. A. M. (2000) Nat. Genet. 26, 265–266). The results show that Gly-41→Arg prevents trafficking of γ but not αβ pumps to the cell surface and abrogates functional effects of γ on αβ pumps. These findings underscore a potentially important role of γ in affecting solute transport, in this instance Mg2+ reabsorption, consequent to its primary effect on the sodium pump.

The Na,K-ATPase, or sodium pump, maintains the high Na+ and K+ gradients across the plasma membrane of animal cells. Accordingly, this pump plays a major role in determining the cytoplasmic Na+ concentration and hence the cytoplasmic concentration of protons and Ca2+, as well as other solutes whose accumulation is driven by secondary countertransport systems. The kinetic properties of the sodium pump are, in turn, subject to complex mechanisms of short- and long-term regulation. While the nature of the catalytic α subunit isoform may be a primary determinant of tissue-specific behavior of the pump, there are also diverse mechanisms underlying pump regulation. (For review, see Refs. 1 and 2).

There is an increasing body of evidence that a family of small, single transmembrane proteins characterized by the motif FXYD are expressed in a tissue-specific manner. To date, at least two members have been identified in kidney, FXYD2 or γ (4, 5) and FXYD4 or the corticosteroid hormone-induced factor, CHIF (6–8). Both modulate the kinetic behavior of the Na,K-ATPase (see Refs. 5, 9, 10–13 for γ and 13, 14 for CHIF). Another related protein, phospholemman-like protein of shark (PLMS) (15), related to FXYD1 (phospholemman) in mammalian heart (16), also modulates function in a phosphokinase C-dependent manner. To date, at least seven members of this family have been identified (3).

The γ subunit of the Na,K-ATPase was discovered over 20 years ago (17, 18) and was shown recently to exist as two major variants in the kidney, γa and γb (19), consistent with predictions based on the Expressed Sequence Tag (EST) data base (20). These are splice variants and differ only in their N-terminal residues. In the rat, the seven N-terminal amino acids TELSANH of γa are replaced by Ac-MDRWYL in γb (19). Expression studies in fetal tissues suggest that a third form may be present (21).

We have previously cloned and expressed the γa and γb variants in mammalian cells and characterized their two main regulatory roles (Refs. 10 and 12 and reviewed in Ref. 22). One function of γ is to increase cytoplasmic K+ antagonism of Na+ activation, which is apparent as an increase in K+Na+ particularly at elevated K+ concentrations. The other function is a γ-mediated increase in the apparent affinity for ATP, concordant with our earlier finding that antibodies raised against the C terminus of γ decreased the affinity for ATP (5). We ascribed the latter decrease in K+ATP to a γ-mediated shift in the poise of the steady-state E1 ↔ E2 equilibrium toward E1. Consistent with this finding is the behavior of both γ subunits expressed in Xenopus oocytes (13). Thus, in the presence but not absence of Na+, both subunits alter the apparent affinity for extracellular K+ in a membrane potential-dependent manner, indicative of a γ-mediated shift in conformational equilibrium toward E1. Although no notable difference between γa and γb function could be detected (12), the significance of the presence of two major variants of γ may be related to their partially overlapping but distinct patterns of expression (12, 29), which, in turn, may be relevant to specific functions along the nephron.

One goal of this study was to gain insight into the structural basis for the two distinct kinetics effects of γ. To this end, we examined the consequences of altering both N- and C-terminal extramembranous regions of γ by deletion and alanine replace-

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‡The abbreviations used are: CHIF, corticosteroid hormone-induced factor; PFO, perfluorooctanoate; PRM, plasma-rich membranes; HDM, high density microsomal membranes; LDM, low density membranes; MES, 4-morpholineethanesulfonic acid; WT, wild-type; NHS, N-hydroxysuccinimide.

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ment of the variant-specific N terminal and deletion of up to ten residues from the C terminus. The other aim was to analyze the functional basis for the transmembrane Gly-41 → Arg mutation associated with familial magnesium-wasting in man (24). This analysis underscores an important role of γ in affecting secondary transport as a result of primary effects on Na,K-ATPase function.

**EXPERIMENTAL PROCEDURES**

**Mutagenesis**—The γ mutants analyzed in the present study were generated as follows. Using the γγ cDNAs as template, a series of PCR reactions was carried out with appropriate primers to generate the cDNAs coding for the deletion of the C-terminal four and ten residues of the γγ variants, γγΔ4 and γγΔ10, respectively, as well as the cDNAs coding for deletion or alteration of the N terminus that is distinct in the two variants, i.e. γNΔ7 (first seven residues deleted) and γN7A (first seven residues replaced by alanines). These mutants are schematized in Fig. 1A. The point mutation in the transmembrane region (Gly-41 replaced by Arg) was introduced into the γγ cDNA using the QuikChange™ site-directed mutagenesis kit (Stratagene).

Expression in Rat α1-HeLa Cells—The above mutated cDNAs were subcloned into the piRES expression vector as described previously (19, 12). All mutations were confirmed by DNA sequencing. The ppiRES expression vector was then transfected into HeLa cells stably expressing the rat α1 subunit of Na,K-ATPase (α1-HeLa cells, kindly provided by Dr. J. B. Lingrel) using the LipofectAmine reagent (Invitrogen) as described (12, 19). Single clones expressing mutated γ were selected in 400 μg/ml hygromycin B. Western blots were carried out to analyze the expression of each mutant.

**Polycrylamide Gel Electrophoresis and Western Blotting**—Unless otherwise indicated, SDS-PAGE was carried out using 10% NuPage gels (Novex) with SDS/MES running buffer. PFO-PAGE was also carried out with 10% NuPage gels in which the detergent perfluorooctanoate (PFO) replaced SDS. For both systems, the running and sample buffers were made according to the recipes supplied by the manufacturer (Novex). Antibodies used were anti-γ (antibody γCS3, described in Ref. 10), anti-γγ, recognizing the N terminus of γ (19), anti-α1 subunit obtained from Sigma (A277), anti-calnexin to recognize the endoplasmic reticulum (StressGen), and anti-giantin to detect the Golgi (a gift from Dr. Edward Chan).

**Subcellular Fractionation**—Transfected HeLa cells were grown to near confluence on 15-cm dishes and fractionated at 4 °C essentially as described by Simpson et al. (25). Briefly, the cells were scraped off the plate, washed twice with ice-cold 20 mM Tris-HCl, 1 mM EDTA, and 255 mM sucrose, pH 7.4 (TES), and then homogenized (30 strokes using a motor-driven teflon pestle/glass homogenizer). Nuclei and unbroken cells were removed by centrifugation at 1,000 × g. The supernatant was then centrifuged for 20 min at 19,000 × g after which the pellet was suspended, layered on a sucrose cushion (1.12 ml sucrose, 20 ml Tris-HCl, 1 ml EDTA, and 1,000 γγ γ) and centrifuged for 60 min at 120,000 × g (Beckman SW32Ti rotor for this and subsequent centrifugations). The membrane-rich fraction at the interface was collected, resuspended in TES, and centrifuged for 30 min at 40,000 × g to obtain a pellet of plasma-rich membranes (PRM). The initial supernatant from the 19,000 × g supernatant was centrifuged at 40,000 × g for 30 min, yielding a pellet of high density microsomal membranes (HDM). The resulting supernatant was then centrifuged at 180,000 × g for 75 min, yielding a pellet of low density membranes (LDM). Each pellet (PRM, HDM, and LDM) was resuspended in 200 μl of TES, and aliquots were taken for the determination of protein concentration (Lowry assay) and Western blot analysis.

**Cell Surface Biotinylation**—The method used is a modification of the method of Stephan et al. (26) used for HeLa cells. Transfected HeLa cells were grown to ~80% confluence in 6-well plates and washed twice with ice-cold PBS/CM (phosphate-buffered saline containing 0.1 mM CaCl₂ and 1 mM MgCl₂). All further steps were carried out on ice. Each well of cells was treated with NHS-S-S-biotin (Pierce; 1.5 mg/ml in 10 μl HEPES, 2 mM CaCl₂, 150 mM NaCl, pH 8.5) for two successive 20 min incubations with gentle shaking. The reagent was freshly prepared for each incubation. After biotinylation, each well was briefly rinsed with PBS/CM, then frozen on dry ice and then treated with the same solution for 30 min on ice to ensure complete quenching of the unreacted NHS-S-S-biotin. The cells were then lysed for 30 min with 500 μl of 1% Triton X-100, 0.1% SDS in L1 buffer (150 mM NaCl, 5 mM EDTA, 50 mM Tris-HCl, pH 7.5, containing 10 μg/ml each leupeptin and pepstatin and 200 μM phenylmethylsulfonyl fluoride). Each sample was then centrifuged at 18,000 × g for 10 min to remove cell debris.

Protein determination on the supernatant (total cell lysate) was performed by the Lowry method. To recover the biotinylated proteins, 100 μg of supernatant protein was incubated with 100 μl of streptavidinagarose beads (Pierce) overnight at 4 °C with end-over-end rotation. The beads were removed by centrifugation, and the supernatant representing the unbound fraction was saved for Western blot analysis. The beads were washed three times with L2 buffer (L1 buffer omitting the SDS), then twice with high salt L2 (L2 containing 500 mM NaCl and 0.1% Triton X-100), and once with 50 mM Tris-HCl, pH 7.5. The biotinylated proteins were eluted from the beads by incubation in 100 μl of SDS-PAGE sample buffer containing 5% β-mercaptoethanol at 37 °C for 5 min.

**Kinetic Assays and Data Analysis**—Kinetic assays of Na,K-ATPase were carried out in triplicate as described previously (12) with either mutant or WT γ-transfected rat α1-HeLa cells assayed concomitantly with mock-transfected rat α1-HeLa cells. As in those previous studies, Kₐₐ and Vₐₐ were obtained by fitting the data to a simple Michaelis-Menten model; Kₐₐ and Vₐₐ were obtained by fitting the data to the 2-site non-cooperative model described by Garay and Gurranah (27) in their classic studies with red cells. The model assumes that Na⁺ ions bind randomly at three equivalent cytoplasmic sites. Unless indicated otherwise, values of Kₐₐ and Kₐₐ were obtained from at least three separate experiments (WT or mutant γ analyzed concurrently with mock-transfected control; compare representative experiments shown in Figs. 2 and 4 carried out with each of at least two different clones of the same wild-type or mutant-transfected α1-HeLa cells. These values were used to quantify the effects of WT and mutant γ subunits as described in Figs. 3 and 5.

**RESULTS**

Expression of Mutants—Our earlier studies with γγ and γγ-transfected HeLa cells stably expressing the rat α1 isoform (α1-HeLa cells) showed that the γ subunit has at least two kinetic effects on Na,K-ATPase: a decrease in Kₐₐ and an increase in Kₐₐ due to K⁺/Na⁺ antagonism. To assess the structural basis for these distinct kinetic effects, extramembranous mutants as well as the intramembranous G41R mutant were constructed and expressed in rat α1-transfected HeLa cells. Several clones with high expression were used for preparation of the membranes. Fig. 1, panel A depicts the constructs, and panels B–D provide verification of their expression following Western blotting of representative clones of each mutant together with rat kidney enzyme, control mock-transfected α1-HeLa, and γγ–α1-HeLa membranes. In the blot shown in panel B, the C-terminal deletion mutants γγΔ4 and γγΔ10 were detected with anti-γγ raised against the N terminus of γγ but not anti-γC. In panel C, mutants with the γγ and γγ distinct residues of the N terminus either deleted (mutant γNΔ7) or replaced by seven alanine residues (γN7A) were detected with anti-γC raised against the C terminus. In all lanes of each blot, similar units of activity were analyzed indicating that the levels of γ expression of the mutants were at least as high as seen in the WT γγ control, which, in turn, is at least as high as that of kidney. It is noted, however, that with the present NuPage system, a species that migrates significantly slower than γγ is seen in the lanes showing γγ and γγ mutants. In our previous studies (19), this species was barely detectable. Its size could reflect the presence of undisassociated γγ dimers using the NOVEX system. To date, we have not pursued this issue further.

The blot in Fig. 1, panel D shows that the G41R substitution in the transmembrane domain of γγ alters the mobility seen in immunoblots. The difference in mobility of the γγ G41R mutant compared with WT γγ is best detected using SDS-PAGE but is clearly seen when electrophoresis is carried out in PFO-PAGE (see “Experimental Procedures”). The reduced mobility probably reflects a structural change due to the charge alteration introduced by the Gly → Arg substitution.
Mutational Analysis of the Na\textsubscript{K}-ATPase γ Subunit

In the present study, several clones of each mutant were analyzed, each paired with control mock-transfected a1-HeLa membranes. Thus, Na\textsuperscript{+} activation profiles determined at 100 mM K\textsuperscript{+} of mutants as well as the wild-type γ\textsubscript{C} or γ\textsubscript{B}-a1-HeLa cells are shown in Fig. 2. Each paired experiment shown is representative of one of several experiments carried out with membranes from several clones of the same mutant. Fig. 3 summarizes the results of all paired experiments (control together with mutant or WT γ). In the presentation of the kinetic effects of each γ mutant relative to WT γ\textsubscript{C} and γ\textsubscript{B}, we have normalized all the data as follows. Values of the ratios of $K_{Na}^\text{C}$ (wild-type γ\textsubscript{C} or γ\textsubscript{B}-transfected a1-HeLa cells) to $K_{Na}^\text{B}$ (control a1-HeLa cells) for all experiments from all clones of the same mutant were averaged, and for each mutant or WT γ the mean ± S.E. are shown. These data show clearly that only the mutation in the transmembrane domain (G41R) abrogated the wild-type γ\textsubscript{C} or γ\textsubscript{B}-mediated decrease in apparent Na\textsuperscript{+} affinity.

In other experiments (not shown), we observed that compared with control mock-transfected cells the aforementioned extramembranous mutants, as well as γ\textsubscript{C} and γ\textsubscript{B}, but not the intramembranous γ\textsubscript{G41R} mutant, increased K\textsuperscript{+} inhibition of activity measured at low Na\textsuperscript{+} concentration (see Fig. 4 in Ref. 12).

**Effect of Mutations on Apparent Affinity for ATP**—The apparent affinity for ATP of each mutant was compared with control a1-HeLa cells in a series of experiments carried out with several clones of each mutant analyzed as described above for determination of $K_{Na}^\text{C}$. Single representative experiments for each mutant are shown in Fig. 4. Fig. 5 summarizes the results of all of the experiments. As for the analysis of effects on $K_{Na}^\text{B}$ for each of the WT or mutant γ subunits the ratio of $K_{ATP}^\text{B}$ (wild-type γ\textsubscript{B} or γ\textsubscript{B}-transfected a1-HeLa cells) to $K_{ATP}^\text{B}$ (control a1-HeLa cells) was obtained, and the mean ± S.E. is presented. As shown previously (12), both γ variants reduce $K_{ATP}$. However, Gly-41 → Arg replacement or deletion of ten or even four of the penultimate C-terminal residues abrogates the γ-mediated increase in ATP affinity. An unexpected finding is the abrogation of the effect on $K_{ATP}$ by removal of the variant-distinct N terminus (γNΔ), particularly since the two γ variants have similar effects despite the notable structural divergence of their N termini.

**Evidence that the Gly-41 → Arg Substitution in the Transmembrane Domain Alters Trafficking of γ to the Cell Surface**—From previous immunolocalization studies, we (12) and others (23) have shown that γ is highly expressed in kidney tubules. In regions where it is present, it is not seen alone but always together with α. In other regions, α is present without γ.

There are three points of evidence that support the theory that the Gly-41 → Arg mutation alters trafficking of γ to the cell surface. The first is indirect and is inferred from the finding that post-translational modification of γ is altered by this mutation. Thus, when a number of different clones of γ\textsubscript{C} or a1-HeLa and γ\textsubscript{G41R} or α1-HeLa were analyzed by Western blotting with anti-γ (Fig. 6), the γ chain appeared as a doublet in γ\textsubscript{C} or a1-HeLa clones, i.e. a lower band shown previously to correspond to γ\textsubscript{C} of kidney and an upper additional species referred to as γ\textsubscript{C}*. In contrast, few, if any, of the γ\textsubscript{G41R} clones showed the additional upper γ\textsubscript{B} band, which is, presumably, a post-translationally modified form of γ\textsubscript{C}.

The second point of evidence for altered trafficking is the difference in distribution of γ\textsubscript{G41R} compared with γ\textsubscript{B} in Golgi-rich membranes. This was apparent when the transfected a1-HeLa cell membranes were fractionated into putatively PRM, HDM, and LDM and then analyzed by Western blotting using anti-α and anti-γC antibodies, as well as anti-calnexin and

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2 $K_{Na}^\text{C}$ and $K_{K}^\text{C}$ are the affinity constants for Na\textsuperscript{+} (extrapolated to [K'] = 0) and for K\textsuperscript{+} at cytoplasmic site(s), respectively. $K_{Na}^\gamma$ is the apparent affinity constant for Na\textsuperscript{+} at cytoplasmic sites.
anti-giantin, as markers of endoplasmic reticulum (Fig. 7, ER) and Golgi, respectively. A representative experiment using this fractionation procedure is shown in Fig. 7. Quantitative densitometry indicated that the relative proportion of γ in the PRM fraction is reduced in γbG41R cells compared with that in WT γb cells, i.e. 25 and 45%, respectively, in the representative experiment shown. Although this rudimentary fractionation precluded good separation of PRM and ER as shown by the abundance of calnexin in both fractions, it is particularly noteworthy that the γ subunit is present in much larger amounts in the Golgi-rich LDM of γbG41R compared with the LDM of WT γb. From quantitative densitometry, the percentage of total γ present in the Golgi-rich LDM fraction was 22.5 ± 3.5% for γbG41R and 6.0 ± 0.7% for WT γb (average of two separate experiments).

The third and most definitive point of evidence is the almost complete absence of mutant γbG41R protein in surface proteins isolated following biotinylation of surface-exposed lysine residues of intact cells. Using the impermeant biotinylation reagent NHS-SS-biotin, this procedure allowed the assessment of the relative amounts of WT γb and γbG41R at the surface of the transfected cells. Following removal of excess reagent and solubilization of the cells, the biotinylated proteins were captured on streptavidin beads and then analyzed by Western blotting with antibodies to γb and γc. Fig. 8 depicts immunoblots of total detergent-solubilized cells (T), biotinylated surface proteins bound to streptavidin beads (B), and material that was unbound (U) to the beads. The total detergent-solubilized and unbound fractions were analyzed in equal amounts with respect to the original cells. For the bound fraction, the amount analyzed was 10 times that of detergent-solubilized and unbound in order to obtain comparable band densities. As shown in Fig. 8, the α subunit appears in surface membranes of both control γb-α1-HeLa and γbG41R-α1-HeLa cells. In contrast, γb appears primarily at the surface, whereas γbG41R remains
inside the cells (unbound fraction) with little, if any, detectable
at the surface.

The much lower proportion of α compared with γ at the cell
surface, as seen by the greater intensity of α in the unbound
fraction (Fig. 8, U), is probably not because of fewer α subunits
at the surface. More likely, the limited accessibility of α lysines
to NHS-SS-biotin results in inefficient biotinylation and conse-
quent underestimation of α subunits at the surface. The results
do, however, permit comparison of the proportion of biotin-
accessible α subunits in γG41R–versus γ-transfected cells.
With this proviso, it is clear that the Gly-41 → Arg mutation
prevents the γ subunit from reaching the cell surface without a
major effect on αβ pump trafficking.

DISCUSSION

The γ subunit of the sodium pump is a member of the FXYD
family of small single-span transmembrane proteins. There are
is intriguing that the two effects of \( \text{CHIF} \) are paradoxically opposite. It is higher affinity for ATP should shift the equilibrium away from \( E_2 \), whereas the \( \gamma \)-mediated increase in \( K^+ \) affinity may counteract and hence minimize the true \( K^+/Na^+ \) antagonism and vice versa. This may come about if \( \gamma \) effects on \( \text{Na,K-ATPase} \) behavior are, in turn, modulated by cell-specific interactions of \( \alpha/\beta/\gamma \) complexes with other cell elements such as those of the cytoskeleton.

The observation that none of the extramembranous mutants abrogated both effects of \( \gamma \) indicates that all of these mutant \( \gamma \) subunits associate with \( \text{Na,K-ATPase} \) \( \alpha/\beta \) dimers. This finding is consistent with the recent report of Beguin et al. (13), which showed that the FYXD motif that is present in these mutants is critical for stable association.

The finding that deletion of the N terminus, like removal of the C terminus (or addition of anti-\( \gamma \)-C-terminal antibodies), abrogates the effect of \( \gamma \) on the \( E_1 \leftrightarrow E_2 \) conformational equilibrium points to long-range effects of \( \gamma/\alpha/\beta \) interactions on \( K^+_\text{ATP} \). Because the N-terminal deletion but not N7A replacement abrogates the \( K^+_\text{ATP} \) effect, the \( \gamma \) effect to stabilize \( E_2 \) does not involve TELSANH or MDRWYL interactions with \( \alpha/\beta \) but rather the remainder of the \( \gamma \) chain.

A physiological basis for the dual effects of \( \gamma \) is that it provides a fine-tuned, self-regulatory mechanism for balancing energy utilization and maintaining appropriate salt gradients across renal epithelial cells. Both \( \gamma \) variants are particularly abundant in the medullary thick ascending limb. As reasoned elsewhere (22), it is in the anoxic regions of the medulla that the increased affinity for ATP effected by \( \gamma \) would serve to maintain pump activity, and the moderate decrease in \( Na^+ \) affinity would serve to balance ATP depletion and maintain an appropriately low intracellular \( Na^+ \) concentration. Accordingly, its dual effect enables \( \gamma \) to imbue the pump with the ability to handle ATP under energy-compromised conditions and yet be self-regulated by having an appropriately modest increase in the \( Na^+ \) concentration set point. Recent studies by Garty et al. (14) have shown that in certain regions with little if any \( \gamma \) in which the apparent affinity for \( Na^+ \) is higher (29), in particular cortical and medullary collecting ducts, the renal pump is associated with \( \text{CHIF} \). CHIF has the opposite effect of \( \gamma \) on \( K^+_\text{Na}^+ \) (13); it increases the apparent affinity for \( Na^+ \) at least 2-fold, which these authors suggest may be critical for aldosterone-responsive tissues, which have an important role in maintaining \( Na^+ \) and \( K^+ \) homeostasis. It is not known yet whether, in mirror image to the \( \gamma \) effect, the increase in apparent \( Na^+ \) affinity effected by \( \text{CHIF} \) reflects a decrease in the apparent affinity for \( K^+ \) acting as a competitive inhibitor of cytoplasmic \( Na^+ \) activation.
An important role of γ in renal cation homeostasis secondary to its association with, and modulation of, Na,K-ATPase is demonstrated by our results showing the functional consequences of mutating Gly-41 to Arg. This study provides evidence that the G41R substitution alters γ interaction with the αβ pump, resulting in the failure of γ both to traffic to the cell surface and to modulate pump kinetics. The former finding confirms the γ routing defect reported by Meij et al. (24). In addition, our experiments indicate that αβ pump trafficking per se is not notably affected.

The significance of the association of renal Mg\(^{2+}\)-wasting with abrogation of γ modulation of Na,K-ATPase is uncertain. It is evident that the consequences of changes in Na\(^{+}\), K\(^{+}\), and Cl\(^{-}\) transport along the different regions of the nephron are varied and complex. Reduced apparent ATP affinity of αβ pumps by abrogation of their modulation by γ may decrease pump activity and lead to secondary changes (reduction) in Mg\(^{2+}\) reabsorption. Accordingly, renal Mg\(^{2+}\)-wasting seen in the dominant hypomagnesemia described by Meij et al. (24) appears to be secondary to the loss of γ modulation of Na,K-ATPase function. The primary cellular mechanism remains to be determined. Also unexplained is the increase in renal calcium absorption and hypocalciuria that is consistently observed in these patients (24).

The experiments described in this study were carried out with cultured apolar cells. The extent to which γ trafficking and abrogation of the γ effects by G41R replacement are different in polar cells remains to be addressed. Current efforts are underway to address this aspect of γ function in polarized renal epithelial cells.

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