Chloride and Potassium Conductances of Mouse Pancreatic Zymogen Granules Are Inversely Regulated by a ≈80-kDa mdr1a Gene Product*

(Received for publication, September 25, 1995, and in revised form, November 20, 1995)

Frank Thévenod§, J an-Peter Hildebrandt¶, J örg Striessnig*, Hugo R. de Jonge*, and Irene Schulzt

From the 111 Department of Physiology, Medical Faculty, University of Saarland, 66421 Homburg/ Saar, Federal Republic of Germany, the 1Department of Biochemical Pharmacology, University of Innsbruck, Peter-Mayrstrasse 1, A-6020 Innsbruck, Austria, and the 2Department of Biochemistry, Medical Faculty, Erasmus University, 3000 DR Rotterdam, the Netherlands

Cl– and cation conductances were characterized in zymogen granules (ZG) isolated from the pancreas of wild-type mice (+/+) or mice with a homozygous disruption of the multidrug resistance P-glycoprotein gene mdr1a (−/−). Cl– conductance of ZG was assayed in isotonic KCl buffer by measuring osmotic lysis, which was induced by maximal permeabilization of ZG membranes (ZGM) for K+ with valinomycin due to influx of K+ through the artificial pathway and of Cl– through endogenous channels. To measure cation conductances, ZG (pH 6.0–6.5) were suspended in buffered isotonic monovalent cation acetate solution (pH 7.0). The pH gradient was converted into an outside-directed H+ diffusion potential by maximally increasing H+ conductance of ZGM with carbonyl cyanide m-chlorophenylhydrazone. Osmotic lysis of ZG was induced by H+ diffusion potential-driven influx of monovalent cations through endogenous channels and nonionic diffusion of the counterion acetate. ZGM Cl– conductances were not different in (−/−) and (+/+0.4 ± 0.2 h–1 compared with (−/−) mice (2.6 ± 0.3 h–1 versus 3.1 ± 0.2 h–1 (relative rate constant)). The nonhydrolyzable ATP analog adenosine 5′-(β,γ-methylene) triphosphate (AMP-PCP) (0.5 mM) activated the Cl– conductance both in (+/+) and (−/−) mice. However, activation of Cl– conductance by AMP-PCP was reduced in (−/−) mice as compared with (+/+0.4 ± 0.2 h–1 versus 7.6 ± 0.7 h–1; p < 0.005). In contrast, ZGM K+ conductance was increased in (−/−) mice as compared with (+/+0.4 ± 0.2 h–1 versus 8.5 ± 1.2 h–1; p < 0.03). In the presence of 0.5 mM AMP-PCP, which completely blocks K+ conductance but leaves a nonselective cation conductance unaffected, there was no difference between (−/−) and (+/+0.4 ± 0.2 h–1 versus 3.2 ± 0.5 h–1). In Western blots of ZG from wild-type mice, a polyclonal MDR1 specific antibody labeled a protein band of ≈80 kDa. In mdr1a-deficient mice, the intensity of this band was reduced to 39 ± 7% of the wild-type signal. This indicates that a mdr1a gene product of ≈80 kDa enhances the AMP-PCP-activated fraction of mouse ZGM Cl– conductance and reduces AMP-PCP-sensitive K+ conductance.

Most members of the superfamily of ATP binding cassette (ABC) transporter proteins act as pumps carrying substrates as diverse as large hydrophobic drugs, small anions, or peptides across membranes at the cost of ATP hydrolysis. Two members of the ABC transporter superfamily, however, have properties of Cl– channels and are also modulated by ATP: the cystic fibrosis transmembrane conductance regulator (CFTR) and the multidrug resistance P-glycoprotein (MDR1). CFTR is responsible for cAMP-activated epithelial Cl– secretion or reabsorption (1) and functions as a Cl– channel that is activated by protein kinase A- and protein kinase C-mediated phosphorylation (2), but activation also requires ATP binding and hydrolysis (3). P-glycoproteins can confer multidrug resistance by actively extruding structurally unrelated, amphiphilic, and hydrophilic drugs from the cells (4). Recently, Higgins and coworkers (5, 6, 11) found that MDR1 overexpression in different cell lines correlates with the appearance of a volume-regulated CI– current. The MDR1 associated CI– current was induced by swelling (5) and required allosteric interaction with ATP or nonhydrolyzable ATP analogs for activation (6). However, the role of MDR1 as a swelling-activated Cl– channel has been challenged (7–10). Recent evidence suggests that MDR1 is not the Cl– channel, but a regulator of a latent channel protein (11). Thus, MDR1 appears to be multifunctional, associated with both transporter and channel regulator activities.

Pancreatic acinar cells secrete NaCl, fluid, and digestive enzymes upon stimulation by secretagogues. In permeabilized pancreatic acini, we have demonstrated that enzyme secretion evoked by the Ca2+ and cAMP signaling pathways depends on the ionic environment of the secretory granules, since it required the presence of isosmotic Cl– and K+ in the medium and was abolished by application of Cl– and K+ channel blockers (12). We therefore postulated that hormonally regulated Cl– and K+ selective channels are present in the membrane of ZG. Upon fusion of ZG with the luminal plasma membrane, the increased influx of salt and water through the granule CI– and K+ channels would promote enzyme secretion (13). Subsequently, we have demonstrated the presence of regulated ion conductance pathways in rat pancreatic ZG membranes. A K+ conductance is inhibited by ATP and nonhydrolyzable ATP analogs and blocked by K+ channel blockers, e.g. quinine and glibenclamide, in a similar manner as ATP-sensitive K+ chan-
phenotype. mdr1b

and

The pellet was resuspended in lysis buffer containing 1 M guanidinium HCl to remove membrane-associated proteins, and membranes were stored in liquid nitrogen. Protein concentration was assayed as described by Bradford (23) using bovine serum albumin as a standard.

Isolation of Brush-border Small Intestinal Plasma Membranes—To prepare mouse brush-border small intestinal membrane vesicles from jejunal and ileal mucosal scrapes, we followed a MgCl2 precipitation protocol as described by Burdkhardt et al. (24).

Assays for Ion Conductances of ZG—Anion and cation conductances of mouse pancreatic ZG were assayed according to a previously reported protocol for quantitative evaluation of macroscopic ion fluxes through endogenous conductance pathways of ZG membranes (13, 14). The absorbance time course on the measurement of ZG which have been resuspended in buffered isosmotic salt solutions, after addition of electrogenic ionophores for membrane permeation of counterions.

Since bulk salt influx into the intragranular space and the resulting granule lysis are limited by the flux of ions through the endogenous conductance pathway, but not by the flux of counterions through the shunt pathways, the slope of the decrease in absorbance with time will represent an estimate of the rate of ions transported through the endogenous conductance pathway.

Anion conductance was measured by resuspending ZG in HEPES-buffered (20 mM, pH 7.0) iso-osmotic (150 mM) potassium salts of the anions tested plus 1 mM EGTA, 0.1 mM MgSO4, and the addition of 5 μM valinomycin, which selectively and maximally permeabilizes ZG membranes to the major cation K+ (13). To measure K+- or nonselective cation conductances, ZG were suspended in 150 mM monovalent cation-acetate solutions containing 1 mM EGTA, 0.1 mM EDTA and buffered with 50 mM imidazole (pH 7.0, adjusted with acetic acid). Since the intragranular pH is about 6.5 (14), an inside-to-outside directed H+ concentration gradient of 65 kDa exists between intragranular space and incubation solution was generated. Cation influx was initiated by addition of 16 μM electrogenic protonophore CCCP, which maximally permeabilizes the granular membrane to H+ and converts the H+ concentration gradient into an inside negative H+ diffusion potential. The inside negative membrane potential, in turn, energizes cation influx through endogenous cation permeabilities. Anion influx occurs through the uncharged monovalent acid, which permeates through the lipid membrane by nonionic diffusion and dissociates to provide the intragranular space continuously with protons for protonation of imidazole as well as for proton efflux from the acidic interior (14). Under these conditions, cation influx through endogenous cation permeabilities is rate-limiting.

Half-times of granular lysis was estimated from the slope of the decrease in absorbance with time between ionophore addition and either experimental half-time or the entire observation period if the half-time was not reached. The absorbance of the suspension was measured at a wavelength of 540 nm in a spectrophotometer at 37°C. The slope of the absorbance change with time was calculated by linear regression of the digitized data. Lysis rates were expressed as half-times of granular lysis or its reciprocal value, i.e. the inverse half-time of lysis, which was considered proportional to the rate constant of lysis. Unless otherwise indicated, data were expressed as means ± S.E. of different preparations. Statistical analysis was carried out with the Statgraphics program using unpaired Student’s t test. Results with less than p < 0.05 were considered significant.

SDS-PAGE and Western Blotting—Electrophoresis and blotting procedures were performed essentially as described earlier (15). Briefly, membrane proteins were separated by SDS-PAGE on 9% acrylamide (Laemmli (25)) minigels and transferred to polyvinylidene difluoride membranes. The efficiency of protein transfer was monitored with prestained protein standards. Blots were blocked with 3% nonfat dry milk in Tris-buffered saline + 0.05% Tween 20 for 6 h and incubated with primary antibody (1:2,000 dilution of the “anti-pgp 389” rabbit polyclonal antibody) overnight. To test the specificity of antibody binding, the anti-ppg 389 antibody was preincubated for 30 min with 1 μM of the epitope peptide sequence KGN...RKE. To detect “gp-300” with a rabbit polyclonal antiserum (1:10,000 dilution), Western blots were probed essentially as described by De Lisse (26) using nitrocellulose membranes for transfer. Following incubation with horseradish peroxidase-conjugated secondary antibodies (1:6,000 dilution), blotting was developed in enhanced chemiluminescence reagents, and signals were visualized on X-ray films. Quantification of chemiluminescence signals was performed on a Bioprofil computer assisted imaging and scanning system (Vilber-Lourmat, Marne La Vallée, France).

RESULTS AND DISCUSSION

To characterize anion conductance of wild-type (+/+) and mdr1a-deficient (−/−) mouse ZG, granules were incubated in different isosmotic K+ salts in the absence (Fig. 1A) or presence (Fig. 1B) of valinomycin (5 μM), which renders the ZG membrane (ZGM) maximally permeable to K+ in. In the presence of valinomycin, granule lysis occurred within seconds, both in

The products of the MDR1 gene in humans and of two genes, mdr1a and mdr1b, in rodents are responsible for the multidrug resistance phenotype.

EXPERIMENTAL PROCEDURES

Materials

AMP-PCP, valinomycin and carbonyl cyanide m-chlorophenylhydrazone (CCCP) were obtained from Sigma. Horseradish peroxidase-conjugated donkey anti-rabbit IgG and enhanced chemiluminescence reagents were purchased from Amersham Corp. The polyclonal “anti-ppg 389” antibody was generated by injecting the peptide KGNLEFRN-VHF5YPSRKE (amino acid residues 389–406 of the human MDR1 sequence) coupled to bovine serum albumin into rabbits and purification of the immune serum on a peptide affinity column (20). Male mice homozygous for a disruption of the mdr1a gene (−/−) (21) and heterozygous (+/−) were kindly provided by Dr. A. H. Schinkel (Division of Molecular Biology, The Netherlands Cancer Institute, Amsterdam, The Netherlands). All other reagents were of analytical grade.

Methods

Isolation of ZG and Purification of ZG Membranes—Zymogen granules were isolated from mouse exocrine pancreas as described earlier (15). Briefly, pancreatic tissue was homogenized using nitrogen pressure cavitation, and ZG were isolated by centrifugation of the immune serum on a peptide affinity column (20). Male mice homozygous for a disruption of the mdr1a gene (−/−) (21) and heterozygous (+/−) were kindly provided by Dr. A. H. Schinkel (Division of Molecular Biology, The Netherlands Cancer Institute, Amsterdam, The Netherlands). All other reagents were of analytical grade.

Isolation of Brush-border Small Intestinal Plasma Membranes—To prepare mouse brush-border small intestinal membrane vesicles from...
Regulation of Zymogen Granule Cl\(^-\) and K\(^+\) Conductances by mdr1a

A

![graph A showing osmotic lysis of pancreatic ZG from wild-type (+/+) or mdr1a-deficient (−/−) mice suspended in iso-osmotic K\(^+\) salts.](image)

B

![graph B showing anion conductance of wild-type (+/+), +/−, or −/− mouse pancreatic ZG. The experimental buffer system was identical to that described in A. Conductance was recorded after addition of the K\(^+\)-selective ionophore valinomycin (5 μM) at the arrow. C, effect of the nonhydrolyzable ATP analog AMP-PCP on Cl\(^-\) conductance of wild-type (+/+) and mdr1a-deficient (−/−) mouse pancreatic ZG.](image)

(+/+) and (−/−) mice, as the result of K\(^+\) influx through the valinomycin pore and of diffusion of the lipophilic anion SCN\(^-\) through the lipid membrane. In the presence of the large anion gluconate, granules from (+/+), (−/−), and (−/+), mice remained equally stable before (Fig. 1A) and after the addition of valinomycin (Fig. 1B) throughout the duration of the experiment. This indicates that the osmotic properties and mechanical stability of the granules, as well as the permeability of the granule membranes to lipophilic and large hydrophilic anions are similar in wild-type and mdr1a-deficient mice.

Whereas ZG incubated in isotonic KCl did not lyse in the absence of ionophore, the addition of the electrogenic K\(^+\) ionophore valinomycin enhanced lysis (Fig. 1B), which indicates that Cl\(^-\) permeates through an anion-selective conductance pathway. Its permeability sequence was I\(^-\) > Br\(^-\) > Cl\(^-\) for both (+/−) and (+/+), mice (not shown), which is similar to that found in rat pancreatic (13) and parotid ZG (27). However, the conductance for Cl\(^-\) (as well as for Br\(^-\) and I\(^-\), not shown) was slightly reduced in (−/−) mice, as compared with (+/+), mice (Fig. 1B). We also investigated Cl\(^-\) conductance in (+/+), and (−/−) mice in the absence and presence of 0.5 mM of the nonhydrolyzable ATP analog AMP-PCP (Fig. 1C). AMP-PCP (0.5 mM), which increases rat pancreatic ZG Cl\(^-\) conductance (15, 17), enhanced lysis both in (+/+), and (−/−) mice. The increase in Cl\(^-\) conductance was, however, more pronounced in (+/+) mice than in (−/−) mice.

To investigate whether the differences in Cl\(^-\) conductance between (+/+) and (−/−) were significant, mean rate constants of ZG lysis from 10–14 experiments were calculated and analyzed by Student’s t test for two independent samples. As can be seen from the left panel of Fig. 3A, mean Cl\(^-\) conductance (expressed as mean rate constant of ZG lysis) in (+/+) mice was 3.1 ± 0.2 h\(^-1\) and 2.6 ± 0.3 h\(^-1\) (mean ± S.E.) in (−/−) mice. This difference was not significant. In the presence of AMP-PCP (0.5 mM), Cl\(^-\) conductance increased to 7.6 ± 0.7 h\(^-1\) in (+/+) mice and to 5.0 ± 0.4 h\(^-1\) in (−/−) mice. This difference was significant (p < 0.005; n = 10). AMP-PCP-sensitive Cl\(^-\) conductance, i.e., the component of the Cl\(^-\) conductance that is activated by 0.5 mM AMP-PCP, was reduced by ~50% in mdr1a-deficient mice, as compared with controls (2.8 ± 0.5 h\(^-1\) versus 5.1 ± 0.7 h\(^-1\); p < 0.02; n = 9). ZG Cl\(^-\) conductance in (−/−) mice was not abolished, which indicated that ZG Cl\(^-\) conductance is affected by disruption of the mdr1a gene, but that mdr1a-independent Cl\(^-\) transport also contributes to ZG Cl\(^-\) conductance.

When granules were incubated in KSCN buffer, granule lysis occurred in the absence of valinomycin and was faster in (−/−) mice than in (+/+) mice (Fig. 1A). For lysis to occur, influx of K\(^+\) and SCN\(^-\) was necessary. Since the difference in lysis between (−/−) and (+/+) mice was no longer present after addition of valinomycin (Fig. 1B), endogenous K\(^+\) conductance pathways, but not SCN\(^-\) diffusion across the ZGM, must have been responsible for the faster lysis in mdr1a-deficient mice. Rat pancreatic ZG contain a K\(^+\)- and RB\(^-\)-selective cation conductance and a nonselective cation conductance in their membrane (14, 15). Therefore, mouse ZG were tested for K\(^+\)-selective and nonselective cation conductances (Fig. 2), as described under “Methods.” As shown in Fig. 2A, K\(^+\) conductance was increased in mdr1a-deficient mice (−/−,CCCP) as compared with mice expressing the mdr1a gene (+/+,CCCP). 0.5 mM AMP-PCP, which blocks rat pancreatic K\(^+\) conductance (14), also reduced K\(^+\) conductance in both (+/+) and (−/−) mice. Even in the presence of 0.5 mM AMP-PCP, K\(^+\) selective conductance was higher in (−/−) mice than in (+/+) mice. The increase of K\(^+\) conductance in mdr1a-deficient mice was more pronounced, since the nonselective cation conductance of ZGM, determined using Na\(^+\) as permeating cation, was not affected by mdr1a gene disruption (Fig. 2B).

A summary of the results from 7–11 different experiments is shown in Fig. 3B. K\(^+\) conductance was increased in (−/−) mice roughly by a factor of 2 (from 8.5 ± 1.2 h\(^-1\) to 14.2 ± 2.0 h\(^-1\); left panel of Fig. 3B). This difference was statistically significant (p < 0.03; n = 11). AMP-PCP (0.5 mM) reduced K\(^+\) conductance in (+/+) mice to 3.2 ± 0.5 h\(^-1\) and to 5.3 ± 0.7 h\(^-1\) in (−/−) mice (Fig. 3B). This AMP-PCP insensitive portion of ZG K\(^+\) conductance, which corresponds to the nonselective cation conductance (15), was not different in (+/+) and (−/−) mice.
Fig. 2. A, Effect of AMP-PCP on K⁺ selective conductance of pancreatic ZG from wild-type (+/+) and mdr1a-deficient (−/−) mice. ZG were suspended in buffered 150 mM potassium acetate with or without 0.5 mM AMP-PCP. At the arrow, the protonophore CCCP (16 μM) was added, where indicated. B, Nonselective cation conductance of wild-type (+/+) and mdr1a deficient (−/−) mouse pancreatic ZG. ZG were suspended in 150 mM sodium acetate. Otherwise, the experimental conditions were identical as described in A.

Fig. 3. Synopsis of Cl⁻ (A) and K⁺ conductance (B) measurements in pancreatic ZG of wild-type (+/+) and mdr1a-deficient (−/−) mice with or without AMP-PCP. Solid bars represent conductance values for wild-type (+/+), and open bars values for mdr1a-deficient mice (−/−). The difference between conditions with and without AMP-PCP corresponds to AMP-PCP activated Cl⁻ and AMP-PCP sensitive K⁺ conductances, respectively. Data are means ± S.E. of 7–14 experiments. p values were calculated using Student's t test for unpaired comparison of condition without (+/+) or with mdr1a disruption (−/−). n.s., not significant.
Regulation of Zymogen Granule Cl⁻ and K⁺ Conductances by mdr1a

(see also Fig. 2B). In contrast, the AMP-PCP-sensitive K⁺ conductance was significantly increased in mdr1a knockout mice (6.5 ± 1.0 h⁻¹), compared with wild-type animals (3.5 ± 0.6 h⁻¹; p < 0.03; n = 7).

From these results we conclude that there is a relationship between mdr1a expression and the activity of ZG Cl⁻ and K⁺ conductance pathways. We therefore correlated ion conductances of wild-type (+/-) and mdr1a deficient (-/-) mice with Western blot analyses of mouse ZGM. We used an affinity-purified rabbit polyclonal antibody against amino acids 389-406 of human MDR1 (anti-pgp 389) to probe electrophoretically separated membrane proteins from ZGM of wild-type or mdr1a-deficient mice. The antibody was used because of low background, high affinity, and specificity toward MDR1 when compared with C219 and JSB-1 antibodies. As shown in the first lane of Fig. 4A, the antibody (dilution 1:2,000) labeled mouse mdr1a, which is the only mdr1 isoform expressed in mouse intestinal brush-border membranes (21). Preincubation of the antibody with 1 µM peptide antigen abolished antibody labeling of the 160-180 kDa band associated with mdr1a, suggesting specificity of antibody labeling (Fig. 4B). In mouse ZGM, the antibody labeled a major band of ~80 kDa (Fig. 4A), a molecular mass that is higher than that found in rat ZGM and could result from differences in glycosylation, the use of different alternative splice sites or may represent a species-specific isofrom. Labeling was abolished by competition experiments with 1 µM peptide epitope (Fig. 4B). In mdr1a-deficient mice, no 160-180 kDa band was detected in intestinal brush-border membranes (second lane of Fig. 4A). In ZGM from mdr1a-deficient mice, the ~80 kDa band was still present, but the signal was much weaker than in the wild-type mice (Fig. 4A, fourth lane). This band was also abolished by preincubation of the antibody with antigenic peptide (Fig. 4B). Signals from four different experiments were scanned, and the intensity of the signals was quantified. Mean intensity of the ~80 kDa band in ZGM from mdr1a-deficient mice was calculated to 39 ± 7% of the wild-type signal (mean ± S.D.). This suggested that a fraction of the protein associated with the ~80 kDa band of ZGM is a mdr1a gene product. Since anti-pgp 389 antibody also labels mdr1b (Western blots of mouse adrenal gland, a tissue that exclusively expresses mdr1b (28); not shown), it is likely that the weak ~80 kDa band detected in ZGM from mdr1a-deficient mice (Fig. 4A) represents an mdr1b gene product. Western blots of ZGM with a polyclonal antiserum to gp300, a sulfated glycoprotein mainly localized to the zymogen granule membrane of mouse pancreas (26), showed signals of comparable intensity in wild-type and mdr1a-deficient mice (Fig. 4C), which indicates that the reduction in intensity of the ~80 kDa band in mdr1a-deficient mice is specific. Western blot analyses of ZGM with the MDR1-specific antibody therefore suggests that both mdr1a and mdr1b gene products are coexpressed in wild-type mouse pancreatic acinar cells and contribute equally to the ~80 kDa band labeled by anti-pgp 389 antibody.

The experiments with ZG isolated from mdr1a-deficient mice demonstrate that disruption of the mdr1a gene partially reduces the ZG Cl⁻ conductance, which is activated by AMP-PCP, and increases AMP-PCP inhabitable K⁺ conductance. As summarized in the model in Fig. 5, we propose that a mdr1a gene product in ZGM is an adenine nucleotide binding protein that regulates both Cl⁻ and K⁺ conductances in a coupled but inverse manner. Further support for our hypothesis is also derived from the reciprocal effects of pharmacological channel modulators, such as AMP-PCP, the sulfonamide derivative glibenclamide, and quinine, which activate rat ZG Cl⁻ and inhibit K⁺ conductance (29). This hypothesis is highlighted by the recent cloning of a pancreatic islet high affinity sulfonylurea receptor, which modulates ATP-sensitive K⁺ channels and turns out to be a novel member of the ABC superfamily of transporters (30). In view of our finding that the increased ZG K⁺ conductance in mdr1a (-/-) remains fully AMP-PCP inhabitable (Figs. 2A and 3B), the K⁺ channel in ZGM, similar to the ubiquitously expressed Kir6.1 K⁺ channel (46), is also likely to interact more directly with AMP-PCP, in addition to its putative interaction with the mdr1a gene product. Furthermore, our results likewise do not allow to discriminate between a model in which AMP-PCP interacts solely with P-glycoprotein, with the Cl⁻ channel, or with both the regulator and the
channel (see Fig. 5).

The results of this study support the (disputed (7–10)) view that there is a regulatory relationship between MDR1 P-glycoprotein gene products and Cl\(^{-}\) channels, as proposed by Hardy et al. (11) for ATP-dependent volume-regulated Cl\(^{-}\) currents and by Luckie et al. (31) for swelling-activated Cl\(^{-}\) and K\(^{+}\) currents. As reported earlier for the P-glycoprotein-regulated volume-sensitive Cl\(^{-}\) current by Gill et al. (6), Cl\(^{-}\) channel activation (as well as K\(^{+}\) channel inhibition) by the mdr1α gene product in ZGM apparently proceeds in the presence of the nonhydrolyzable ATP analog AMP-PCP, implying that P-glycoprotein interaction with the ion channels does not require ATP hydrolysis, in clear contrast to its activity as a drug pump.

There is increasing evidence that ABC transporters may regulate ion channel function as well. Both MDR1 and CFTR have structural homology (32), share common functions as Cl\(^{-}\) (2, 5) and ATP-channels (33, 34), and exhibit a multidrug resistance phenotype (35). There is no doubt that CFTR operates as a Cl\(^{-}\) channel (36). However, it is interesting to note in the context of our conclusions that cystic fibrosis is associated with a decreased activity of an outwardly rectifying Cl\(^{-}\) channel (37–40) and an increased open probability of amiloride-sensitive Na\(^{+}\) channels (41, 42). These examples and our results emphasize the possibility that MDR1 and CFTR gene products, including potential splicing variants (43–45), may be responsible for the regulation of different ion channels.

Acknowledgments—We thank Drs. A. H. Schinkel and P. Borst (Division of Molecular Biology, The Netherlands Cancer Institute, Amsterdam, The Netherlands) for the generous gift of mdr1α deficient and wild-type mice, and Dr. R. C. De Lisle (Department of Anatomy and Cell Biology, University of Kansas Medical Center) for providing antisera to go300.