

Expression and Developmental Regulation of Na⁺,K⁺ Adenosine Triphosphatase in the Rat Small Intestine

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Abstract

The Na⁺,K⁺-ATPase ion pump plays a critical role in fluid and electrolyte physiology of the small intestine. Here we show that, of the three known α isotypes ($\alpha 1$, $\alpha 2$, and $\alpha 3$) of the sodium pump found in the rat, only $\alpha 1$ is expressed in the small intestine. The expression of this isotype, considered at the level of mRNA, is under developmental control, with the adult intestine exhibiting approximately a threefold increase in $\alpha 1$ message over the neonate. Cortisone treatment of the neonate results in near-adult levels of $\alpha 1$ mRNA expression. An increase in the abundance of $\alpha 1$ isotype parallels the changes in its mRNA expression. β subunit mRNA is expressed coordinately with the $\alpha 1$ subunit mRNA. A four- to five-fold rise in the Na⁺,K⁺-ATPase activity is also developmentally induced. (*J. Clin. Invest.* 1992. 90:1016–1022.) Key words: sodium pump • ion transport • glucocorticoid • isozymes • gene expression

Introduction

Na⁺,K⁺-ATPase (EC 3.6.1.3) is an $\alpha\beta$ heterodimer located in the plasma membrane of animal cells (1, 2), which is responsible for maintaining Na⁺ and K⁺ gradients across plasma membranes. A 100,000-D α catalytic subunit peptide acts to transport three Na⁺ ions out of the cell and two K⁺ ions into the cell for each molecule of ATP hydrolyzed. A 55,000-D β subunit is a glycoprotein that may be necessary for the assembly of the sodium pump into the plasma membrane (3, 4).

Na⁺,K⁺-ATPase serves a vital function in various mammalian tissues. In the nervous system, the pump establishes the resting membrane potential necessary for signal propagation. In the heart Na⁺,K⁺-ATPase affects the force of muscle contraction by generating a Na⁺ gradient, which in turn affects the concentration of Ca²⁺ in muscle cells. In the kidney the ion pump regulates water reabsorption and venous blood volume by controlling Na⁺ transport across the renal tubule into the bloodstream. Finally, in the intestine, Na⁺,K⁺-ATPase, which is located in the basolateral membrane, creates the driving force for ion and solute transport across the epithelium.

In the rat there are three highly homologous isotypes of the α subunit, $\alpha 1$, $\alpha 2$, and $\alpha 3$, and each is encoded by different genes (5). The cDNA identity for these isoforms reaches 76% homology, whereas the amino acid sequence has > 85% homology (6). The isotypes differ in their affinity for substrates (7, 8) and for ouabain (9–11), which can inhibit the pump by interfering with ATP hydrolysis. Thus far, all three isotypes are expressed only in the brain; but in other tissues a subset of the α isotypes has been found (12). One β subunit derived from a variety of alternatively spliced mRNA species is expressed in most tissues (13, 14), although other isotypes of the subunit have also been found in select tissues (15, 16).

In the enterocyte the electrochemical gradient established by Na⁺,K⁺-ATPase has a twofold effect. Under physiological conditions, the sodium gradient facilitates the absorption of fluid and ions from the lumen across the epithelial cell into the circulation. Under pathophysiological conditions, such as the interaction of cholera toxin and other bacterial toxins with their membrane receptors (e.g., ganglioside GM₁) or the stimulation by other secretagogues, the pump also prevents the excessive loss of water and ions into the intestinal lumen. It is generally accepted (17) that cholera toxin-induced cAMP accumulation opens the chloride channel in the apical membrane of crypt cells, permitting mucosal Cl[−] secretion. cAMP stimulation also inhibits the reabsorption of NaCl in villus cells by blocking the apical membrane Na⁺/H⁺ and Cl[−]/HCO₃[−] exchangers. Na⁺,K⁺-ATPase is unaffected by this toxin and continues to pump sodium ions across the basolateral membrane into the blood stream.

Oral rehydration therapy (18) used in patients with secretory diarrhea makes use of the principle that a salt and glucose solution can enhance the osmotic reabsorption of water across the intestinal epithelial cell. This therapy requires an operational sodium pump in the basolateral membrane and a Na⁺/glucose cotransporter in the apical membrane of enterocytes. Since the sodium pump can counteract the altered intestinal ion flux across the intestine due to cholera toxin, we hypothesize that the difference in host sensitivity to cholera toxin between immature and mature animals may be related in part to the altered density of the sodium pump in the basolateral membrane.

In previous studies we have demonstrated an increased sensitivity to cholera toxin-induced diarrhea in the small intestine of immature compared with mature animals (19). In this study we show that the enzyme pump is indeed more active and more abundant in the adult than in the neonatal intestine. We also provide evidence that the isotype for Na⁺,K⁺-ATPase responsible for this activity is regulated at the level of gene expression, and that cortisone, a hormone closely associated with intestinal maturation (20), increases the expression of the pump.

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Methods

Animals and cortisone injections. Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) were housed in an animal room with a 12-h light/dark cycle, fed rat chow (Purina, St. Louis, MO) and allowed water ad libitum. 2-wk-old rats were considered to be neonates; 8-wk-old rats were considered to be adults. In the cortisone injection experiments, each litter of suckling rats was divided into two groups. One group of animals was injected intraperitoneally with a single dose (5 mg/100 g body weight) of cortisone acetate (Merck Sharp & Dohme, West Point, PA) in a 0.1-ml saline suspension at 9 d of age. Another group was injected with saline alone (0.9% NaCl) and used as controls. The animals were allowed to remain with their mothers until they were 2 wk old.

Nucleic acid probes. cDNA probes corresponding to three rat α subunits and one β subunit of Na^+, K^+ -ATPase were used. $\alpha 1$ cDNA used in the Northern blot analysis was a rb5 1.2 kbp clone (21). $\alpha 2$ and $\alpha 3$ cDNAs came from Ea5/z11 (2.9 kbp) and rb13c (2.7 kbp) clones, respectively (22). In constructing the S1 probe (Fig. 1), an $\alpha 1$ clone was cut with Xho 1 restriction endonuclease (New England Biolabs, Beverly, MA) and subcloned into the Sal 1 restriction site in the pUC 19 polylinker. The 623-bp probe was generated with a double-digest Bam H1/Afl 3. Successful hybridization with RNA would then result in a 246-bp fragment after S1 digestion. $\alpha 2$ and $\alpha 3$ cDNA clones were both cut with Aha 2 restriction endonuclease and cloned into the Acc 1 restriction site in the pUC 19 polylinker. 734- and 556-bp probes, respectively, were generated with Afl 3 restriction endonuclease. The expected protected fragment for $\alpha 2$ was 357 bp whereas for $\alpha 3$ it was 179 bp. Rb 19, originally constructed with ~900 bases of Na^+, K^+ -ATPase β subunit cDNA in the laboratory of Dr. R. Levenson (Yale University, New Haven, CT) (14), was used in a Northern blot analysis of the β subunit mRNA. These cDNA probes were body-labeled using random hexamers as primers (Pharmacia LKB, Piscataway, NJ), [α - ^{32}P]dCTP, 3000 Ci (111 TBq)/mmol (Du Pont NEN, Boston, MA), unlabeled deoxynucleotides (Pharmacia LKB), and the cloned Klenow fragment of DNA polymerase I (Amersham Inc., Arlington Heights, IL), according to the method of Feinberg and Vogelstein (23).

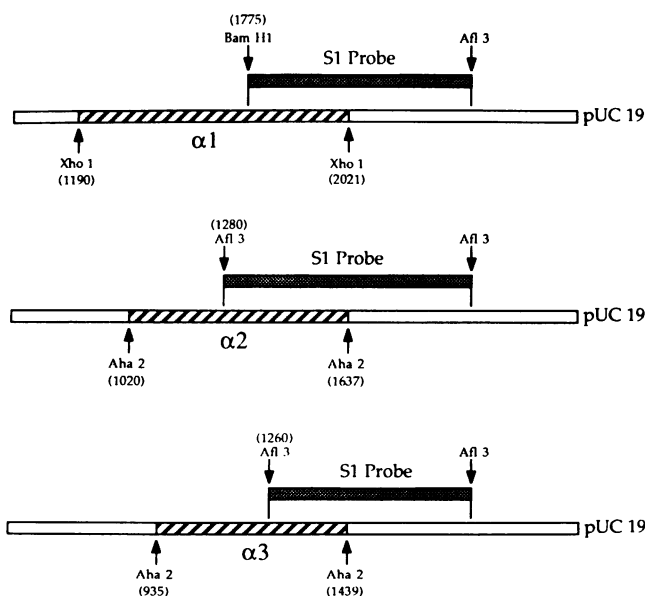


Figure 1. The construction of probes for the S1 nuclease protection assay. The lengths of the probes (in bp) were: $\alpha 1$, 623 (initial), 246 (protected); $\alpha 2$, 734 (initial), 357 (protected); and $\alpha 3$, 556 (initial), 179 (protected).

Monoclonal antibodies. Monoclonal antibodies were a gift of Dr. K. J. Sweadner (Harvard Medical School, Boston, MA). McK1, McB2, and McB-X3 were specific for $\alpha 1$, $\alpha 2$, and $\alpha 3$ Na^+, K^+ -ATPase isotypes, respectively. The first two antibodies belong to a mouse IgG1 subclass, the latter to a IgM class (24).

Na^+, K^+ -ATPase activity. The following procedures were done at 4°C. Mucosa was scraped from the proximal and distal portions of the rat small intestine and homogenized with 9 vol of buffer (pH 6.8) containing the following (mM): 100 Tris-HCl, 1 EDTA, 25 imidazole, and 0.1% deoxycholic acid. The homogenate was centrifuged at 3,000 g for 15 min, and the resulting supernatant was then centrifuged at 105,000 g for 1 h. The resulting pellet was resuspended in 100 mM Tris-25 mM imidazole (pH 6.8) by homogenization and used for the enzyme assay immediately. The assay mixture, in a final volume of 0.1 ml, contained the following (mM): 100 NaCl, 20 KCl, 3 MgCl_2 , 3 ATP (Tris salt), 10 imidazole (pH 6.8), and the enzyme solution (50–100 μg protein) with or without 1 mM ouabain. The reactions were carried out at 37°C for 15 min and stopped by the addition of ice-cold 15% trichloroacetic acid. The phosphorus released was determined by the method of Chen et al. (25). Na^+, K^+ -ATPase activity was calculated from the difference between the activities measured in the presence or absence of 1 mM ouabain. The protein concentration was determined by the method of Lowry et al. (26).

Total RNA preparation. Proximal and distal small intestine was removed from decapitated 2-wk- and 8-wk-old rats and immediately frozen in liquid nitrogen. The intestine was pulverized in a frozen state and total RNA was extracted according to a modified guanidine-thiocyanate procedure of Chirgwin et al. (27). The RNA was pelleted through a CsCl gradient, resuspended in the following (mM): 10 Tris-HCl, 1 EDTA, pH 7.9 buffer. The concentration of each sample was determined based on the absorbance at 260 nm (A_{260}). The purity of each sample was determined based on the ratio of A_{260} to A_{280} ($A_{260}:A_{280}$).

Northern blot analysis. Total RNA (10 μg) from each intestinal sample was denatured with formamide, separated according to size on an agarose-formaldehyde gel, and transferred onto a synthetic membrane (GeneScreen+; Du Pont NEN) using standard capillary blot techniques (28). Each membrane was prehybridized overnight at 42°C, probed overnight with $1\text{--}2 \times 10^7$ cpm cDNA at 42°C, and washed to a final stringency of $0.2 \times \text{SSC}/0.1\%$ SDS at 65°C. The blots were subsequently exposed to preflashed Kodak XAR-5 film for 1–24 h at -80°C with an intensifying screen. Some autoradiographs were analyzed on a densitometer (Ultrascan XL; Pharmacia LKB).

S1 nuclease protection. The 4-bp overhang generated at the 5' end of each probe with restriction endonuclease was filled in using a cloned Klenow fragment of DNA Polymerase I (Amersham) and four radiolabeled nucleotides for 30 min under conditions recommended by the manufacturer. Labeled products were passed through a Sepharose CL-6B matrix (Pharmacia LKB) to remove unincorporated nucleotides, precipitated with 1 μl of 20 mg/ml glycogen (Boehringer Mannheim Biochemicals, Indianapolis, IN) as carrier, 0.3 M NaOAc, pH 4.8, and 2 vol of ethanol, washed with 70% ice-cold ethanol, and lyophilized until dry. The probes were subsequently resuspended in hybridization buffer (80% deionized formamide, 0.4 M NaCl, 0.04 M Pipes pH 6.4, 1 mM EDTA) to a final activity of 5000 cpm/ μl of solution. Lyophilized total RNA (10 μg) or transfer RNA (tRNA) was resuspended thoroughly in 10 μl hybridization solution, denatured at 90°C for 10 min, transferred immediately to a 55°C water bath, and allowed to hybridize overnight, completely submerged. The samples remained at 55°C until 300 μl ice-cold S1 digestion buffer (0.28 M NaCl, 0.03 M NaOAc pH 4.8, 4.5 mM ZnSO_4 , 20 $\mu\text{g}/\text{ml}$ sheared salmon sperm DNA, 667 U/ml S1 Nuclease [Boehringer Mannheim Biochemicals], previously diluted to 33.3 U/ μl in 4°C storage buffer [0.1 M ZnSO_4 , 0.1 M NaOAc]) was added to each tube. Each sample was subsequently removed from the water bath, rapidly but gently mixed, chilled on ice, and incubated at 37°C for 40 min. Digestion was stopped with 75 μl of termination buffer (2.5 M NH_4OAc , 0.05 M EDTA) and the samples

were precipitated with glycogen as a carrier in 400 μ l isopropanol on dry ice, pelleted, lyophilized, and resuspended in 15 μ l of loading buffer (80% deionized formamide, 1 \times TBE, xylene cyanol, bromphenol blue). The samples were heated to 90°C, cooled on ice, and 7–8 μ l of each sample was loaded onto a preelectrophoresed 8 M urea, 1 \times TBE, 5% polyacrylamide 20-cm gel. The gel was run at < 25 mA, dried, and exposed to x-ray film at –80°C for 24–72 h with intensifying screen. The counts associated with each band were collected on a radioanalytic imaging system (AMBIS Systems Inc., San Diego, CA). Vacuum-dried gels were analyzed for 6 h.

Enterocyte isolation and particulate fraction preparation. Enterocytes were isolated from the proximal rat small intestine according to a modified method described by Weiser (29). A protease inhibitor PMSF (1 mM final concentration) was added to all the buffers before use. An intestinal sac was filled with the buffer and incubated at 4°C. Enterocytes from adult rats were collected every 20 min for four consecutive periods and pooled together. The collecting time was reduced to 15 min for neonatal enterocytes. The following procedures were done at 4°C: isolated enterocytes were homogenized in a homogenizing buffer (0.1 M Tris-HCl, pH 7.4) containing 1 mM PMSF, 20 μ g/ml aprotinin, and 10 μ g/ml leupeptin as protease inhibitors. After centrifugation of the homogenate at 3,000 g for 15 min, the supernatant was centrifuged at 105,000 g for 1 h. The resulting pellet was resuspended in homogenizing buffer and used in immunoblotting.

Gel electrophoresis and immunoblotting. The particulate fraction of enterocytes in sample buffer was subjected to SDS-PAGE on a 5% polyacrylamide gel, under conditions described by Laemmli (30). Proteins were electrophoretically transferred to nitrocellulose (31). Immunoblotting was performed as described (24, 32). Filters were incubated with each of the three antibodies for 1 h (McK1, 1:20 dilution; McB2, 1:20; and McB-X3, 1:1), washed, and then incubated for 1 h with alkaline phosphatase-conjugated antibodies (Sigma Chemical Co.) raised against mouse IgG and IgM as appropriate. The alkaline phosphatase-conjugated second antibody was detected by the indoxyl phosphate/nitro blue tetrazolium method of Blake et al. (33). Protein bands were quantitatively analyzed on a laser computing densitometer (model 300S; Molecular Dynamics, Sunnyvale, CA).

Results

Developmental differences and cortisone induction of Na^+, K^+ -ATPase activity. The ontogeny of Na^+, K^+ -ATPase activity in the proximal and distal portions of the rat small intestine is shown in Fig. 2. Although no significant regional difference was noted, the enzyme activity was clearly affected by the age of the animal. In 8-wk-old rats this activity was four to five times higher than in 2-wk-old suckling rats. The activity increased threefold between the second and third postnatal week. This rise in enzyme activity coincided with weaning, a critical period in the maturation of the small intestine.

To test the role of glucocorticoids in regulating Na^+, K^+ -ATPase activity, we injected 9-d-old rats once with cortisone. The animals were killed 5 d later at 2 wk of age. Cortisone-treated animals exhibited a three- to fourfold higher Na^+, K^+ -ATPase activity than the untreated controls in both regions of the gut (Fig. 3).

Northern blot analysis of mRNA expression. Total RNA was collected from adult, neonatal, and cortisone-treated neonatal intestine and tested for the presence of the three Na^+, K^+ -ATPase α and one β isotypes with radiolabeled cDNA probes. Transcripts corresponding to the $\alpha 1$ and β subunit genes were abundant in all samples (Fig. 4A). The pattern of expression of these subunits was age dependent, with the 8-wk-old intestine showing higher levels of the $\alpha 1$ and the β

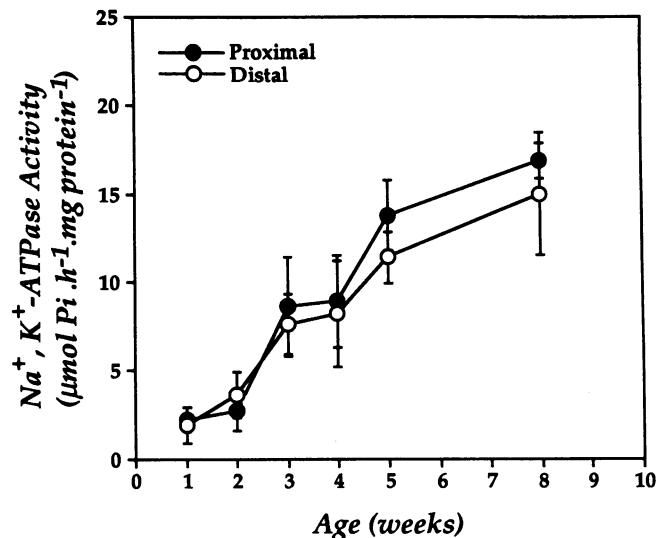


Figure 2. Postnatal development of Na^+, K^+ -ATPase activity in the rat small intestine. Each point represents a mean \pm SE of four samples, prepared from three to four rats per sample for 1–2 wk and one rat per sample for 3–8 wk.

subunit transcripts than the 2-wk-old intestine. Samples from cortisone-treated intestine also exhibited increased expression of these subunits relative to the neonate. The analysis of autoradiographs on a densitometer revealed an increase of two- to threefold for adult $\alpha 1$ message and an increase of approximately twofold for message from cortisone-treated intestine compared with that from the neonatal sample (Fig. 4B). A parallel but smaller increase was also noted in the β message (Fig. 4B). These findings suggested that the expression of Na^+, K^+ -ATPase $\alpha 1$ and β mRNAs is developmentally regulated and that cortisone can increase this expression. The tran-

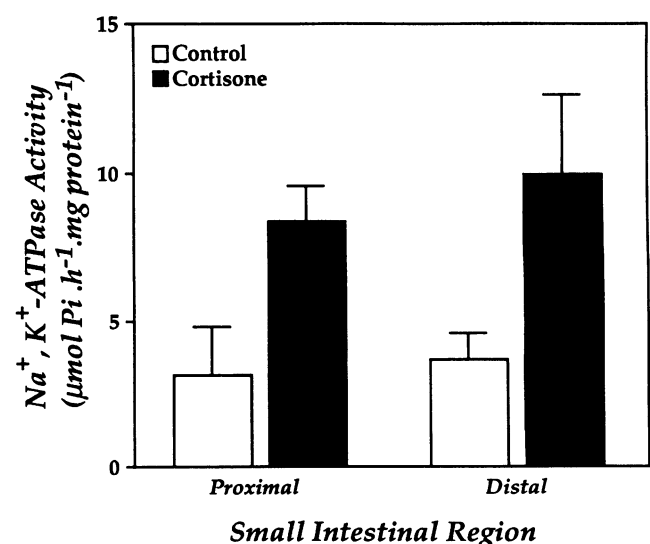


Figure 3. Effect of cortisone treatment on Na^+, K^+ -ATPase activity in the rat small intestine of 2-wk-old suckling rats. Each bar represents a mean \pm SE of four intestinal samples, prepared from three rats per sample. See Methods for cortisone treatment.

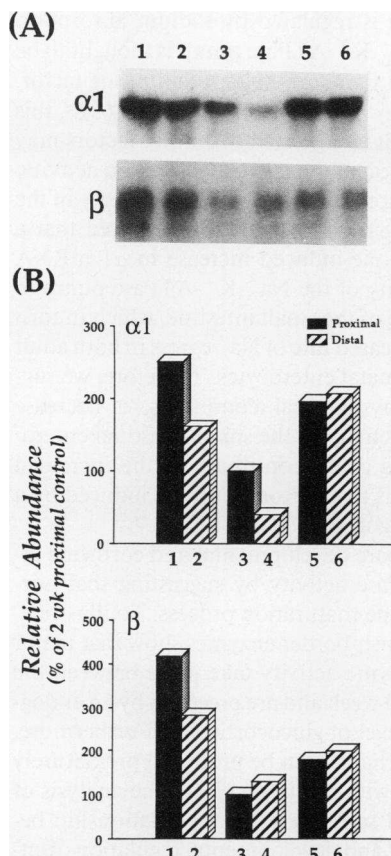


Figure 4. Northern blot analysis of Na^+, K^+ -ATPase mRNA. Total RNA was prepared from the proximal (lanes 1, 3, and 5) and distal (lanes 2, 4, and 6) small intestine of adult (lanes 1 and 2), neonatal (lanes 3 and 4), and cortisone-treated neonatal (lanes 5 and 6) rats. The intestine from 4 rats 8 wk of age and 8–10 rats 2 wk of age was pooled for each RNA sample. The blot containing 10 μg RNA per lane was analyzed successively with $\alpha 1$ and β subunit cDNA probes, exposed for 4 h ($\alpha 1$) or overnight (β) at -80°C with an intensifying screen. (A) Northern blots and (B) relative mRNA abundance. Each bar represents a mean of two determinations.

scripts of the remaining α subunits ($\alpha 2$, $\alpha 3$) could not be consistently detected in the intestine by Northern blot analysis.

S1 nuclease protection analysis of mRNA expression. To determine whether $\alpha 2$ and $\alpha 3$ were indeed expressed and to obtain more quantitative data about the changes in $\alpha 1$ expression, we used a S1 nuclease protection analysis. RNA from intestinal samples, rat brain, and tRNA was tested for the presence of the three Na^+, K^+ -ATPase α isotypes with appropriate cDNA probes (Fig. 5 A). Correct fragments corresponding to each of the three isotypes were protected from S1 digestion with rat brain RNA. No fragment was detected with tRNA, used here as a negative control. In intestinal RNA, only $\alpha 1$ transcript was found. Furthermore, the level of $\alpha 1$ mRNA was differentially expressed during development and after cortisone treatment (Fig. 5 B). When measured as counts per relevant band, there was approximately a three-fold increase in $\alpha 1$ message in adult RNA samples and a 2.5-fold increase in cortisone-treated samples compared with the neonatal samples (Fig. 5 C).

Immunodetection of Na^+, K^+ -ATPase isozymes. The analysis of intestinal membrane proteins and rat brain controls with isozyme-specific monoclonal antibodies yielded predictable results (Fig. 6 A). All three α -isozymes of Na^+, K^+ -ATPase were identified in the brain microsomal samples, but only $\alpha 1$ -isozyme protein was found in the intestinal epithelium. Furthermore, $\alpha 1$ isozyme was prominent in the adult and cortisone-treated membrane fractions (Fig. 6 B). There was approximately a 1.5–1.8-fold increase in the $\alpha 1$ -isozyme protein level in both groups of samples compared with the neonatal samples (Fig. 6 C).

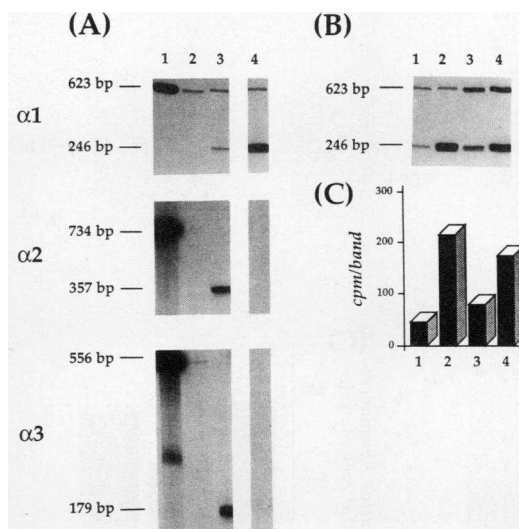


Figure 5. S1 nuclease analysis of intestinal Na^+, K^+ -ATPase α -isotype mRNAs. (A) Analysis of the proximal small intestinal samples of adult rats with $\alpha 1$, $\alpha 2$, and $\alpha 3$ isotype-specific probes. One-tenth of the probe added to RNA during hybridization step (see Methods) is shown in lane 1; 100 μg tRNA (lane 2) and 5 μg brain RNA (lane 3) were used as controls; and 10 μg total RNA from the adult proximal small intestine (lane 4) was used in each sample. The gels were exposed to preflashed x-ray film overnight ($\alpha 1$ blot) or for 4 d ($\alpha 2$ and $\alpha 3$ blots) at -80°C with an intensifying screen. (B) Comparison of $\alpha 1$ mRNA expression in the proximal intestine of adult (lane 2), neonatal (lane 3), and cortisone-treated neonatal (lane 4) rats (shown on a representative blot). 10 μg total brain (lane 1) and intestinal rat RNA were used. The gel was exposed to x-ray film overnight at -80°C with an intensifying screen. In some instances, the excessive DNA probe reannealed when the cold S1 nuclease was added, giving rise to a protected 623-bp band. The reannealing of excessive probe had no bearing on the DNA/RNA hybrids formed during the incubation of probe with the total RNA. (C) The gel in panel B was analyzed on a radioanalytic system for 6 h. The same area around the 246-bp band in each sample was integrated. The columns represent total counts per minute in each area. Each bar represents a mean of two determinations.

Discussion

Previous attempts to describe the regulation of the Na^+, K^+ -ATPase activity have evoked a complex scheme of control mechanisms. The activity of Na^+, K^+ -ATPase is a function of several α and β isotypes of the pump's subunits, which combine in equal ratios to form the active membrane complex. These isotypes are independently and differentially expressed in mammalian tissues. The α isotypes vary in their substrate and ouabain affinities. The β isotypes, which have not been assigned a catalytic role, are believed to influence the pump's activity through their contribution to the stability of the enzyme complex. Ontogeny, hormones, and nutritional conditions are some of the circumstances that may alter the expression of Na^+, K^+ -ATPase isotypes in mammalian tissues. The possible basis for the enzyme regulation by these factors could be a modification in gene expression and/or a change in the stability of mRNAs and protein isotypes. In this study we set out to determine how the activity of intestinal

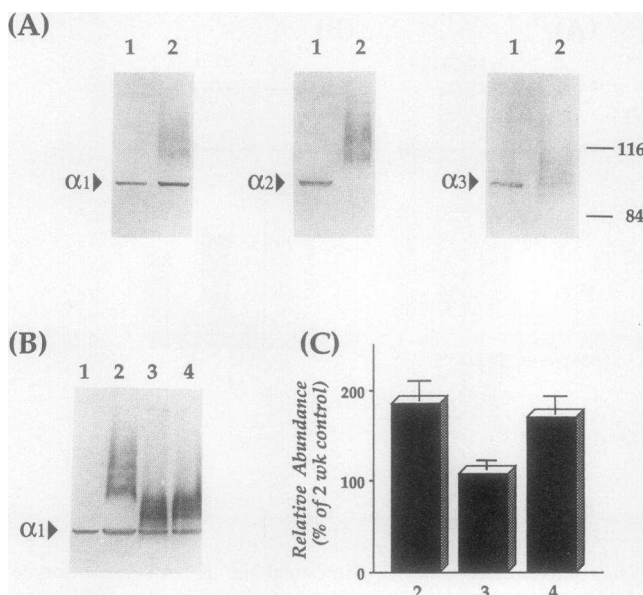


Figure 6. Immunoblot analysis of Na^+, K^+ -ATPase α isoforms. (A) Detection of $\alpha 1$, $\alpha 2$, and $\alpha 3$ isoforms in adult rat brain and proximal small intestine with their respective monoclonal antibodies, McK1, McB2, and McB-X3. Each lane contained 75 μg membrane protein. Lane 1, brain microsome; lane 2, small intestine. (B) Comparison of $\alpha 1$ isoform in the proximal small intestine of adult (lane 2), neonatal (lane 3), and cortisone-treated neonatal (lane 4) rats. The brain microsome (lane 1) was used as the control. Molecular mass references (in kD) are as indicated. The larger, diffuse bands are nonspecific due to the cross-reaction between rat intestinal samples and second antibodies. (C) Relative abundance of $\alpha 1$ -isozyme proteins shown in panel B. Each bar represents a mean \pm SE of three determinations.

Na^+, K^+ -ATPase is regulated and to compare its developmental regulation to hormonal induction of this activity.

We had several reasons to believe that Na^+, K^+ -ATPase activity is regulated both developmentally and by glucocorticoid hormones. In a previous study (19) we have reported that neonatal rats were more susceptible to cholera toxin than either adult rats (50-fold) or cortisone-treated neonates (1,000-fold). This increase in sensitivity to cholera toxin by neonatal rats could not be explained by receptor binding or density but appeared to be associated with a three- to fivefold increase in Na^+, K^+ -ATPase enzyme activity between newborn and adult and cortisone-treated neonatal groups (19). Other studies have shown that rat ileum pretreated with glucocorticoids absorbed more fluid than untreated controls (34) and that rabbits deficient in glucocorticoids exhibited depressed Na^+ absorption and increased Cl^- secretion (35). This present study confirmed our earlier findings. In the small intestine of rats, treated as initially described (19), Na^+, K^+ -ATPase activity rose threefold in response to cortisone treatment and four- to fivefold from 2 to 8 wk of age. Furthermore, the data in this study demonstrate a differential Na^+, K^+ -ATPase isotype gene expression, not only at the mRNA level but also at the protein level, and these changes were well correlated with the altered enzyme activity.

The current dogma suggests that the Na^+ pump rate in the enterocyte is determined solely by its intracellular sodium con-

centration, which in turn is regulated by sodium absorption into the cell (36). The Na^+, K^+ -ATPase pump is thought to be present in abundance and therefore not a rate-limiting factor. However, in a recent review by Schultz and Hudson (37), this dictum is challenged and it is suggested that other factors may be involved in the regulation of the overall Na^+ pump activity. One of the major factors appears to be the pump density in the basolateral membrane (38). In this study, we showed that a developmental and hormone-induced increase in $\alpha 1$ mRNA resulted in a greater density of the Na^+, K^+ -ATPase pump in the basolateral membrane of the small intestine, which in turn might account for the increased rate of Na^+ egress in both adult and cortisone-treated neonatal enterocytes. Therefore, we suggest that under pathophysiological conditions, a decrease Na^+, K^+ -ATPase pump activity in the small intestinal enterocytes of neonatal animals could contribute to the increased sensitivity of these animals to secretory diarrhea induced by a cholera toxin-like secretagogue.

It is tempting to compare developmental and cortisone induction of Na^+, K^+ -ATPase activity by suggesting that hormone treatment mimics the maturation process. To illustrate, studies of rat intestinal brush-border enzymes show that major ontogenic changes in enzyme activity take place between the second and third postnatal week and are preceded by an endogenous rise in the serum level of glucocorticoids. Furthermore, the same developmental change can be produced prematurely by treating neonatal rats with cortisone (20). The analysis of gene expression has shed some light on the relationship between cortisone treatment and developmental regulation of enzyme activity. In an adipose cell line, dexamethasone treatment led to the early accumulation of differentiation-specific gene products (39). The same study confirmed that glucocorticoids did not stimulate gene expression beyond the level expected in the absence of the hormone, suggesting that cortisone, in this system at least, affected the rate by which changes took place, not the degree of change.

We tested the expression of Na^+, K^+ -ATPase isotype mRNAs to determine whether at this level the developmental and hormonal induction of activity appeared similar. An earlier survey of ontogenic expression of Na^+, K^+ -ATPase isoforms in a variety of rat tissues showed that a complex pattern of isotype induction, among $\alpha 1$, $\alpha 2$, and $\alpha 3$, characterized most tissues (40). Northern blot analysis of total intestinal RNA from neonatal, cortisone-treated neonatal, and adult rats initially exhibited an equally complex pattern of different isotype expression. The pattern of expression in the cortisone-treated intestine did not correspond to that of adult intestine (data not shown). However, a more sensitive S1 nuclease analysis of mRNA expression detected only $\alpha 1$ mRNA in all samples tested. (The extensive homology shared by Na^+, K^+ -ATPase isoforms made the identification of specific isoforms with cDNA probes alone very unreliable; nevertheless, the changes in $\alpha 1$ expression as determined by both procedures were comparable.) This isotype mRNA was much more abundant in the adult rat intestine than in the neonatal intestine and approximated adult levels in cortisone-treated neonatal intestine. The analysis of membrane proteins with isotype-specific antibodies substantiated these findings: only $\alpha 1$ could be detected in membrane fractions and the abundance of pumps increased in the course of development and hormone treatment. Recently,

Giannella et al. (41) have conformed in a preliminary report that only the $\alpha 1$ -isotype mRNA is expressed in the intestinal epithelial cells. They performed Northern blot analysis with isotype-specific cDNA probes that were different from those used in this study.

Based on our data, cortisone-induced changes in the intestinal Na^+, K^+ -ATPase expression appears to mimic ontogenic changes. An additional body of evidence suggests that these changes in enzyme activity take place at the level of gene expression. Such evidence is consistent with the general understanding that cortisone regulates gene expression through glucocorticoid response elements specific for promoters of various genes (42). In all cases where Na^+, K^+ -ATPase expression was altered by glucocorticoids and developmental factors, the levels of α and β mRNA increased together and proportionally to the number of pumps expressed on the cell surface. Examining α and β mRNA isotype levels in other rat tissues during development, Orlowski and Lingrel (40) found that an increase in expression of α subunits coincided with an increase in the expression of the β subunit, with $\alpha 1$ and β levels most closely coordinated. The analysis of rat colonic Na^+, K^+ -ATPase (43) revealed that $\alpha 1$ and β subunit mRNA expression was low at birth, but reached adult levels by day 25. Furthermore, glucocorticoid treatment of adult rats resulted in a short-term increase of both $\alpha 1$ and β mRNA expression 2 h after hormone injection. The former findings are very similar to the Northern blot analysis results in the current study, which show the $\alpha 1$ and β subunit mRNA levels increasing together during intestinal development. In addition, we describe a comparable increase in the abundance of Na^+, K^+ -ATPase $\alpha 1$ -isozyme protein levels in the rat small intestine. Such information was not available previously.

In conclusion, the Na^+, K^+ -ATPase activity in the rat small intestine is determined by the expression of the $\alpha 1$ and β subunits, but not by other α isotypes. Protein expression of $\alpha 1$ induction is correlated with its mRNA expression during intestinal development and by injection with glucocorticoid hormones.

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