Na^+-K^+-ATPase gene expression in rat intestine and Caco-2 cells: response to thyroid hormone

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Giannella, Ralph A., John Orlowski, M. Lynn Jump, and Jerry B. Lingrel. Na^+-K^+-ATPase gene expression in rat intestine and Caco-2 cells: response to thyroid hormone. *Am. J. Physiol.* 265 (*Gastrointest. Liver Physiol.* 28): G775–G782, 1993.—Expression of the Na^+-K^+-adenosinetriphosphatase (ATPase) gene family in rat intestinal epithelial cells was examined using RNA blot hybridization analyses. Rat intestinal epithelial cells express only the α_1- and β_1-subunit mRNAs. A gradient in expression of α_1- and β_1-subunit mRNA was seen along the villus-crypt unit in both jejunum and ileum, i.e., villus tip >> crypt cells. Regional differences in expression were observed along the intestine. α_1- and β_1-subunit mRNA abundance was similar in jejunum, ileum, and colon while enzymatic activity was highest in the jejunum and lowest in the ileum. Administration of thyroid hormone to thyroidectomized rats increased the expression of α_1- and β_1-subunit mRNAs in jejunum but not in colon. Hypothyroidism had no effect on subunit mRNA expression. The human intestinal cell line Caco-2 was also studied. These cells also expressed only the α_1- and β_1-isoform mRNAs and demonstrated a developmental profile in both mRNA and enzymatic activity. Furthermore, in Caco-2 cells both α_1- and β_1-mRNAs and Na^+-K^+-ATPase enzymatic activity were stimulated by thyroid hormone. Caco-2 cells transfected with 5′ flanking regions of the human Na^+-K^+-ATPase β_1-gene linked to the chloramphenicol acetyltransferase (CAT) reporter gene responded to 3,5,3′-triiodothyronine (T_3) treatment with increased expression of CAT activity. This suggests that the 5′ flanking region of the β_1-gene contains a thyroid hormone response element and that T_3 upregulation occurs at the transcriptional level. The Caco-2 cell system may be an excellent model to study Na^+-K^+-ATPase gene expression in the intestine.

sodium ion-potassium ion-adenosine triphosphatase; Caco-2; thyroid hormone; intestine

SODIUM ION-potassium ion-adenosinetriphosphatase (Na^+-K^+-ATPase; EC 3.6.1.3) is a heterodimeric transmembrane protein found in all mammalian cells. This protein is responsible for the ATP-dependent transmembrane exchange of Na^+ for K^+ and is responsible for maintaining the transmembrane Na^+ gradient. Because three Na ions are exchanged for two K ions, this exchange results in the establishment and maintenance of a transmembrane electrical potential. The transcellular Na^+ gradient provides the energy for many active transport processes (13).

The heterodimer is composed of an 112-kDa α-subunit and a highly glycosylated 55-kDa β-subunit. The α-subunit contains the ATP-, Na^+-, and K^+-binding sites and is the receptor for cardiac glycosides (13). The function of the β-subunit is uncertain, although recent evidence supports a role for the β-subunit in the assembly and transport of the $\alpha\beta$-dimer to the cell surface (17). Recent studies have revealed the existence of three isoforms of the α-subunit (α_1, α_2, and α_3; see Ref. 25) and at least two isoforms of the β-subunit (β_1 and β_2; 15). Each of these subunits is the product of a separate gene. The tissue distribution and regulation of the various subunit isoform mRNAs and protein have been studied extensively in nonintestinal tissues and have been shown to be regulated by the hormonal and ionic mileau (for review, see Ref. 20).

Although a great deal is known about the function of Na^+-K^+-ATPase in the intestine, little is known about the molecular biology of this gene family in this tissue. Two recent studies have demonstrated that in the rat small intestine and colon only the α_1- and β_1-subunit mRNAs are expressed and that the levels of expression may be regulated by glucocorticoids (6, 30).

Accordingly, the purpose of this study was to identify which isoforms of Na^+-K^+-ATPase are expressed in the epithelium of the rat intestine and to determine their proximal to distal distribution along the intestinal tract, their distribution along the small intestinal villus-crypt unit, and the responsiveness of these subunit mRNAs to thyroid hormone. Furthermore, to determine whether the human intestinal cell line Caco-2 might serve as a model to study this gene family, Na^+-K^+-ATPase gene expression was characterized in this cell. Lastly, data are presented on the responsiveness of Na^+-K^+-ATPase mRNAs and enzymatic activity to various hormones.

MATERIALS AND METHODS

Reagents. Dowex AG 1-X8 resin was obtained from Bio-Rad (Melville, NY), tissue culture media and fetal calf serum were from GIBCO (Grand Island, NY), and restriction enzymes and T_4 polynucleotide kinase were from Promega (Madison, WI). [α-^{32}P]dCTP and [γ-^{32}P]dATP were obtained from Amersham (Arlington Heights, IL), and D-threo-[*dichloroacetyl*-1,2-^{14}C]-chloramphenicol was from Du Pont-New England Nuclear (Boston, MA). Hormones, charcoal, and other reagents were obtained from Sigma Chemical (St. Louis, MO).

Animals. Adult Sprague-Dawley rats were obtained from Charles River Breeding Laboratories and thyroidectomized, and sham-thyroidectomized Sprague-Dawley rats were obtained from Zivic-Miller Laboratories (Zellenople, PA). Operated rats were allowed to recover for 14 days before further manipulation. Thyroidectomized rats were than divided into two groups: those given daily injection of L-thyroxine (0.1 $\mu g \cdot g$ body $wt^{-1} \cdot day^{-1}$ sc) and those given vehicle alone. Sham-operated rats were also injected with vehicle alone. Animals were killed 3 days after injections were begun, and intestines were harvested. Serum measurement of total and free T_4 confirmed the thyroid hormone status of each animal. All rats were housed on a 14 h light:10 h dark schedule, and Purina rat chow and water were available ad libitum.

Intestinal cells and tissues. In most studies, isolated cells obtained from the small and large intestine were used. Isolated small intestinal enterocytes were obtained from jejunum and ileum by the sodium citrate method of Weiser as previously described (3). Five fractions from the villus tip to the crypt were obtained. Sucrase activity was measured in each individual cell population. Total RNA was also isolated from each fraction. The nature of the isolated cells was documented by sucrase assay (3, 7) and by light microscopy. Isolated colonocytes were also obtained from rat large intestine by the method of Roediger and Truelove (21). These cells were obtained from the upper one-half of the colonic glands as documented by light microscopy.

Whole mucosal scrapings prepared from jejunum, ileum, and colon were used to compare the proximal-distal expression of Na^+-K^+-ATPase activity and mRNA levels. Mucosa was scraped with a glass slide, and a portion was homogenized in 40 ml of sucrose buffer [(in mM) 250 sucrose, 10 triethanolamine, 0.1 phenylmethylsulfonyl fluoride, pH 7.6) for 70 s with the use of a Tekmar tissuemizer. The homogenate was centrifuged for 10 min at 2,500 *g*. The supernatant was filtered through two layers of cotton gauze and centrifuged for 20 min at 19,500 *g*. The pellet was resuspended in sucrose buffer and assayed for Na^+-K^+-ATPase activity. The remaining portion of mucosa was used to extract total RNA. To assess the effect of thyroid hormone status, whole mucosal scrapings were also prepared from jejunum and colon of thyroidectomized and sham-operated rats, and RNA was extracted.

Cell culture. Caco-2 cells, a human intestinal cell line originally derived from a carcinoma of the colon but having many of the features of small intestinal enterocytes (20), were kindly provided by Dr. Alain Zweibaum (Institut National de la Santé et de la Recherche Médicale, Villejuif Cedex, France). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum. Media were changed on Mondays, Wednesdays, and Fridays. Cells were subcultured weekly after trypsinization and split 1:5 into 75-cm^2 flasks, 25-mm tissue culture wells, or 100-mm tissue culture plates as needed. Unless otherwise specified, experiments were performed 14–16 days after plating, 10–12 days after cells had reached confluence.

In studies examining responsiveness of Na^+-K^+-ATPase to various hormones, cells were grown and maintained in DMEM media supplemented with 10% fetal calf serum, which had been treated with charcoal and the anion exchange resin AG 1-X8. This procedure removes steroid and thyroid hormones (23), which we documented by monitoring the disappearance of a trace quantity of added [^{3}H]estradiol and by assay of 3,5,3′-triiodothyronine (T_3) and T_4. To assess the effect of various hormones on Na^+-K^+-ATPase activity and subunit isoforms, cells were cultured in "hormone-depleted" media to which different hormones were added and were harvested 14 days later. In T_3 time course studies, cells were split into "hormone-depleted" media. On *day 10*, T_3 was added, and cells were harvested 0–48 h later.

Enzymatic assays. Sucrase activity in Caco-2 cell membranes was measured as previously described (3, 7), and Na^+-K^+-ATPase activity was measured by the K^+-stimulated neutral phosphatase assay as described by Esman (5). Caco-2 cell membranes were prepared as follows: cells were harvested from flasks in 3 ml of 5 mM tris(hydroxymethyl)aminomethane (Tris), pH 7.6, utilizing a rubber policeman. Ten milliliters of sucrose buffer was added, and cells were homogenized for 10 s with a Tekmar tissuemizer and centrifuged at 12,500 *g* for 20 min. The pellet was washed once and suspended in 5 mM Tris for assay. Membranes were subjected to three cycles of freeze-thaw immediately before assay to ensure that all vesicles were ruptured.

Na^+-K^+-ATPase activity in rat membranes, prepared as described above, was measured using a continuous spectrophotometric enzyme-linked assay as described previously (19). Membranes were exposed to 0.02% sodium dodecyl sulfate (SDS) for 10 min before assay to ensure that all vesicles were ruptured. Protein concentrations were determined using Bio-Rad Protein Assay Reagent.

RNA isolation, Northern, and RNA slot-blot analysis. Total cellular RNA was isolated by the guanidine isothiocyanate method described by Chomczynski and Sacchi (4). Northern blots were prepared as follows: total cellular RNA (20 μg) was denatured with glyoxal, fractionated on 1% agarose gels, and blotted onto Nytran membranes by capillary transfer. The membranes were baked, prehybridized, and hybridized according to the manufacturer's protocol (50% formamide, 5× sodium chloride-sodium phosphate-EDTA (SSPE), 5× Denhardt's solution, 200 μg/ml salmon sperm DNA, and 0.1% SDS). RNA (5 μg) for slot-blot analysis was treated similarly. A Schleicher & Schuell Minifold II Slot-Blotter apparatus was used. Isoform-specific cDNA restriction endonuclease fragments for α_1-, α_2-, α_3-, and β_1-subunits were prepared and used for analysis of rat mRNAs, as previously described (18). For hybridization analysis of human Caco-2 mRNA, subunit-specific oligonucleotide probes, 60 bases in length, were synthesized from regions of the cloned α- and β-isoform human cDNA sequences. Under the hybridization and wash conditions used, these probes were isoform specific. Rat cDNA probes were radiolabeled with [α-^{32}P]-dCTP using the random primer method. Oligonucleotide probes were end-labeled with [γ^{32}P]dATP using T_4 polynucleotide kinase. After completion of hybridization, blots were washed in 2× sodium chloride-sodium citrate (SSC), 0.1% SDS at 25°C for 30 min followed by 0.1× SSC, 0.1% SDS at 65°C for 30 min. Autoradiography was performed at −70°C with Kodak XAR-5 film (Eastman Kodak, Rochester, NY) using a Du Pont Kronex Lightning Plus intensifying screen (Du Pont-New England Nuclear). The relative levels of the subunit mRNAs were quantitated by densitometry using a Hoefer Scientific Instruments scanning densitometer as previously described (18). α_1- and β_1-signal intensities were normalized by signals resulting from hybridization with an 18S oligonucleotide probe specific for ribosomal RNA (14).

Construction of human β_1 chimeric genes. The gene for the human Na^+-K^+-ATPase β_1-subunit has been characterized previously (11). A 1.6-kb genomic fragment corresponding to the 5′ flanking region of the human β_1-gene (1141 to +491 nucleotides; +1 = transcription start site; see Ref. 11), labeled Hβ_1-1141, was linked to a reporter gene, the bacterial chloramphenicol acetyltransferase gene (CAT). Expression plasmids for Caco-2 transfection studies were constructed as follows: the CAT gene was inserted into the *Hind* III-*Bam*H I sites of the plasmid pBluescript KS. Hβ_1-1141 was inserted into this plasmid at a *Sal* I site in the polylinker region 5′ to the CAT gene insert and called Hβ_1-1141CAT. In addition, a smaller Hβ_1-CAT gene chimera (Hβ_1-327CAT) was constructed by deleting the first 814 bp of the Hβ_1-1141CAT gene using the restriction endonuclease *Xho* I. These chimeric genes were transfected into Caco-2 cells and were then cultured for 48 h in the absence or presence of T_3. Cellular extracts were prepared from these cells and assayed for CAT activity.

Transfection experiments. Caco-2 cells were transfected with the CAT-containing plasmids using the calcium phosphate-DNA coprecipitation method (1). Caco-2 cells were cultured into 100-mm tissue culture dishes and fed with DMEM media containing 10% hormone-depleted fetal calf serum that had been treated with activated charcoal and anion-exchange resin

Dowex AG 1-X8. After 24 h, cells were transfected with 15 μg plasmid DNA containing the $H\beta_1$/CAT constructs and 5 μg of a plasmid pRV110 containing the β-galactosidase gene. The latter was used to correct for transfection efficiencies. After 24 h incubation, the media were changed to media containing T_3 (50 nM), and cells were harvested 48 h later and assayed for CAT and β-galactosidase activities as described (1). CAT activity was calculated as percent conversion of the substrate chloramphenicol, after normalizing values with β-galactosidase activity.

Data presentation. All experiments were performed at least three times. For slot-blot analysis, a minimum of three autoradiographic signals was evaluated for each data point. All data are expressed as means ± SE. Statistical significance was assessed by use of the Student's *t* test.

RESULTS

Expression of Na^+-K^+-ATPase subunit mRNAs in rat intestine. As shown in Fig. 1, only the α_1- and β_1-isoforms were detected. The α_2- or α_3-mRNAs were not detected in any intestinal sample, although both α_2- and α_3-mRNAs were detected in brain, which served as a control tissue. When rat intestinal samples were hybridized with the β_1-isoform cDNA probe, several β_1-RNA transcripts were detected, a situation similar to that seen in other tissues (29).

Expression of Na^+-K^+-ATPase mRNAs along the villus-crypt unit. Enterocytes isolated from both jejunum and ileum demonstrated a gradient in sucrase activity from villus tip to crypt as expected. In the jejunum there was a 17-fold fall in sucrase activity from villus tip to crypt (74.4 ± 31.4 vs. 4.3 ± 2.3 $\mu mol \cdot min^{-1} \cdot g\ protein^{-1}$, respectively). In the ileum, there was a ninefold fall (19.7 ± 5.0 vs. 2.3 ± 2.3 $\mu mol \cdot min^{-1} \cdot g\ protein^{-1}$, respectively). Total cellular RNA was prepared from epithelial cells isolated from the villus/crypt unit of jejunum and ileum and subjected to Northern blot analysis. A gradient in mRNA expression was seen along the villus-crypt axis in both jejunum and ileum (Fig. 1). Both α_1- and β_1-subunit mRNAs exhibited greatest expression in mature cells at the top of the villi (fractions 1 and 2), with significantly reduced levels found in fractions obtained from more immature cells toward the crypts. Hybridization of the RNA with an oligonucleotide probe to 18S RNA revealed that all lanes were loaded approximately equally, with the exception of jejunum, *lane 1*, which was somewhat underloaded. These changes were characterized further by slot-blot analysis, and the hybridization intensities were quantitated by densitometry (Fig. 2). In the jejunum, there was an approximately fivefold fall in α_1-mRNA and an approximately five- to sevenfold fall in β_1-mRNA, from villus tip to crypt, respectively. In the ileum, there was a three- to fourfold fall in both α_1-mRNA and β_1-mRNA from villus tip to crypt.

Expression of Na^+-K^+-ATPase along the intestine. The distribution of Na^+-K^+-ATPase mRNA abundance and enzymatic activity along the proximal-distal extent of the intestine are shown in Table 1. α_1- and β_1-subunit mRNA abundance was higher in ileum and colon than in jejunum, but this was not statistically significant. In contrast, Na^+-K^+-ATPase enzymatic activity demonstrated an entirely different pattern. Activity was highest in the jejunum and lowest in the ileum with the colon intermediate (jejunum > ileum, $P < 0.04$; colon > ileum, $P < 0.03$).

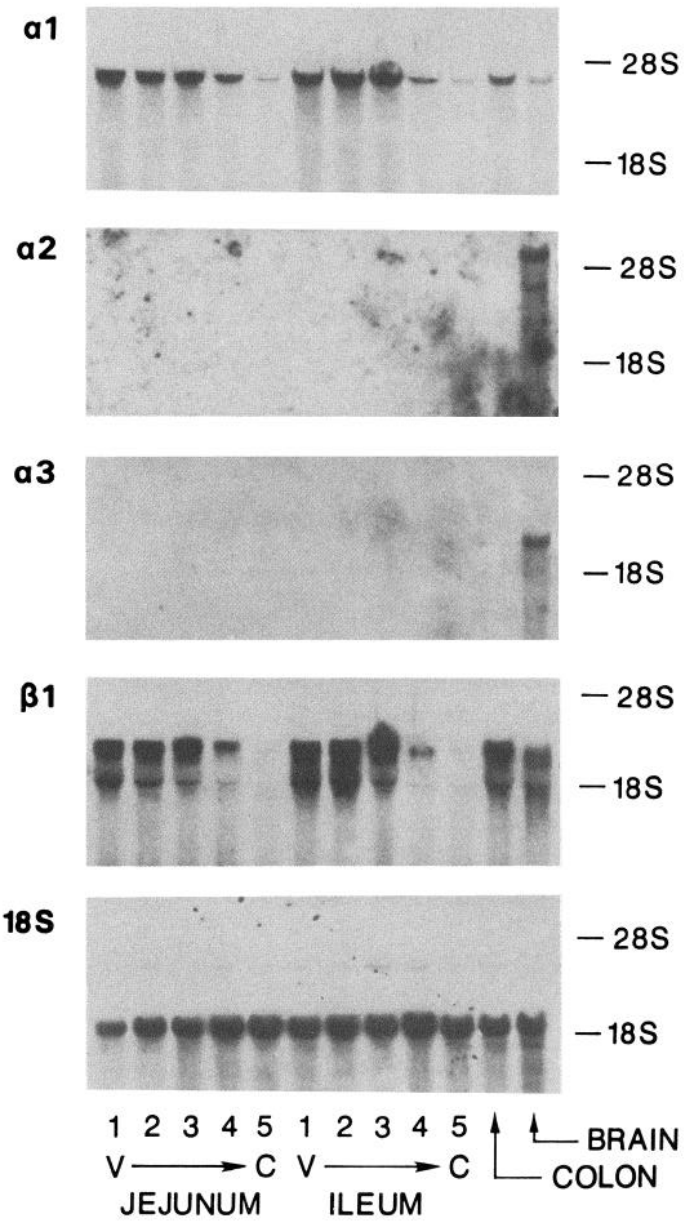

Fig. 1. Northern blot analysis of Na^+-K^+-ATPase α- and β-isoform mRNAs in rat intestinal epithelial cells. Each lane contains 20 μg of total cellular RNA isolated from isolated intestinal cells. Five epithelial cell fractions were isolated consecutively from the tip of the villus (V) to the crypt (C) (*1–5*) of both jejunum and ileum as described in MATERIALS AND METHODS. A single fraction of colonocytes was similarly isolated. The last lane contains rat brain RNA, which serves as a positive control for hybridization to the α-isoform and β_1-subunit cDNA probes. The positions of 28S and 18S RNA are indicated. *Bottom*: same blot hybridized with an oligonucleotide probe specific for 18S ribosomal RNA.

Response of rat intestinal Na^+-K^+-ATPase mRNAs to thyroid hormone. The effect of alterations in thyroid hormone status on Na^+-K^+-ATPase subunit mRNA abundance in rat jejunum and colon is shown in Table 2. Hyperthyroid animals had a 2.7-fold and 2.8-fold increase in α_1-and β_1-mRNA abundance, respectively, in the jejunum compared with euthyroid controls. Hypothyroid ani-

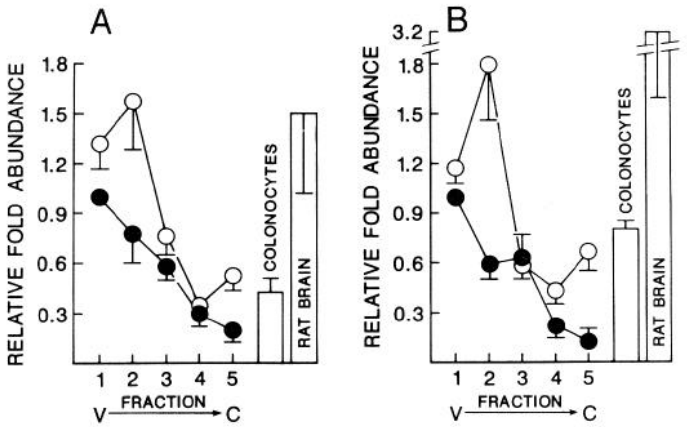

Fig. 2. Quantitation of Na^+-K^+-ATPase mRNA abundance in rat intestinal epithelial cells. RNA samples are the same as those shown in Fig. 1 (*A*: α_1-mRNA abundance; *B*: β_1-mRNA abundance). Bars, mRNA abundance in colonocytes and rat brain for comparison. Total cellular RNA (5 μg) from each sample was subjected to slot-blot analysis. Hybridization intensities were quantitated by densitometry and then normalized to intensities obtained with hybridization using an oligonucleotide probe to 18S ribosomal RNA, which accounted for minor differences in loading samples. A value of 1 was arbitrarily assigned to the level of mRNA seen in fraction 1 of jejunum, and the levels of the other fractions were normalized to that value. ○, jejunum; ●, ileum. All analyses were repeated at least 3 times, and data are presented as means ± SE. Experiment was performed 3 times with similar findings.

Table 1. *Na^+-K^+-ATPase subunit mRNA abundance and enzymatic activity along the intestine*

	mRNA Abundance		Enzymatic Activity
	α_1	β_1	
Jejunum	0.84±0.10	0.59±0.08	47.87±16.30
Ileum	1.35±0.37	0.84±0.25	3.11±2.41
Colon	1.19±0.44	0.73±0.14	20.30±4.77

All data are means ± SE of 3 rats. mRNA abundance is expressed as densitometric ratios of α_1/18S or β_1/18S. Na^+-K^+-ATPase activity is expressed as micromoles per hour per milligram of protein.

Table 2. *Effect of thyroid hormone status on Na^+-K^+-ATPase α_1- and β_1-mRNA abundance in rat jejunum and colon*

	Jejunum		Colon	
	α_1	β_1	α_1	β_1
Hypothyroid	0.20±0.02	0.18±0.06	0.57±0.04	0.28±0.08
Euthyroid	0.17±0.04	0.25±0.09	1.16±0.73	0.66±0.54
Hyperthyroid	0.45±0.18*†	0.71±0.11‡§	0.78±0.22	0.34±0.14

All data are means ± SE of 4 rats. mRNA abundance is expressed as densitometric ratios of α_1/18S or β_1/18S. * $P < 0.02$ compared with euthyroid animals; † $P < 0.03$ compared with hypothyroid animals; ‡ $P < 0.0005$ compared with euthyroid animals; § $P < 0.0001$ compared with hypothyroid animals.

mals did not demonstrate any significant change in either α_1 or β_1 jejunal mRNA abundance compared with euthyroid controls. In contrast to the jejunum, colonic α_1- and β_1-mRNA abundance were not altered by thyroid hormone status.

Expression of Na^+-K^+-ATPase mRNAs in human intestinal Caco-2 cells. To develop a model system to characterize the regulation of Na^+-K^+-ATPase gene expression in the intestine, we selected the well-characterized human intestinal cell line Caco-2, which has been shown to possess many of the morphological and biochemical features of the normal small bowel enterocyte and to undergo developmental maturation with time in culture (20).

Total RNA was isolated from Caco-2 cells at various intervals after plating and was examined by RNA blot analysis. As shown in Fig. 3, the α_1- and β_1-isoforms of Na^+-K^+-ATPase were detected in these cells. α_2- and α_3-mRNAs were not detected in this cell line, when oligonucleotide probes specific for these isoforms (data not shown) were used. Thus the profile of Na^+-K^+-ATPase isoform expression is similar to the profile seen in rat intestine.

Developmental expression of Na^+-K^+-ATPase mRNAs in Caco-2 cells. The α_1- and β_1-mRNAs also demonstrated a developmental pattern of expression (Fig. 4). The abundances for both mRNAs were lowest 2 days after cell plating and increased to peak levels at ~9 days of culture and then decreased moderately thereafter. RNA slot-blot analysis and quantitation by densitometry (Fig. 4*A*) demonstrated that α_1-mRNA abundance was augmented fourfold from *days 2* to *9* in culture and then decreased thereafter. A similar pattern was observed for β_1-mRNA abundance. Na^+-K^+-ATPase activity (Fig. 4*B*) paralleled

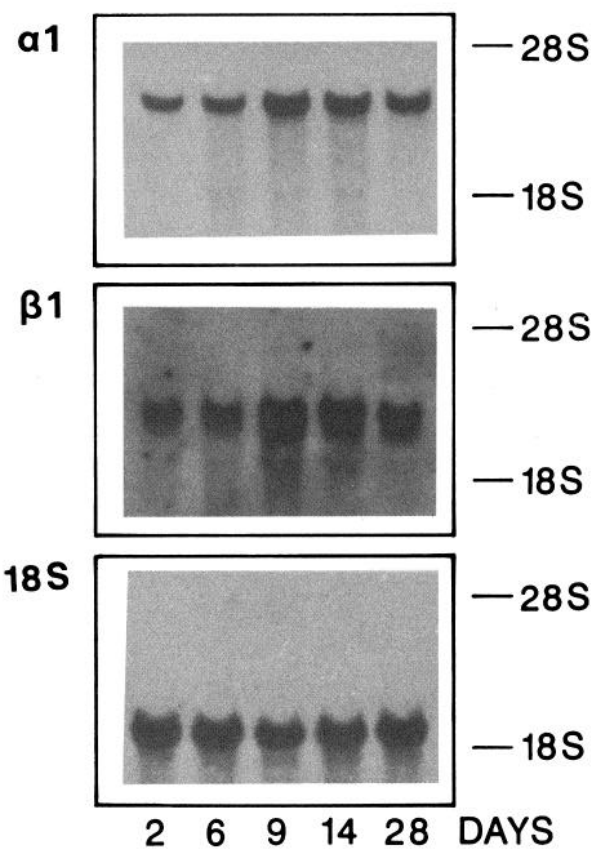

Fig. 3. Northern blot analysis of the Na^+-K^+-ATPase α_1- and β_1-isoform subunit mRNAs in Caco-2 cells of various ages. Each lane contains 20 μg of total cellular RNA. Numbers at bottom represent no. of days in culture after plating of cells.

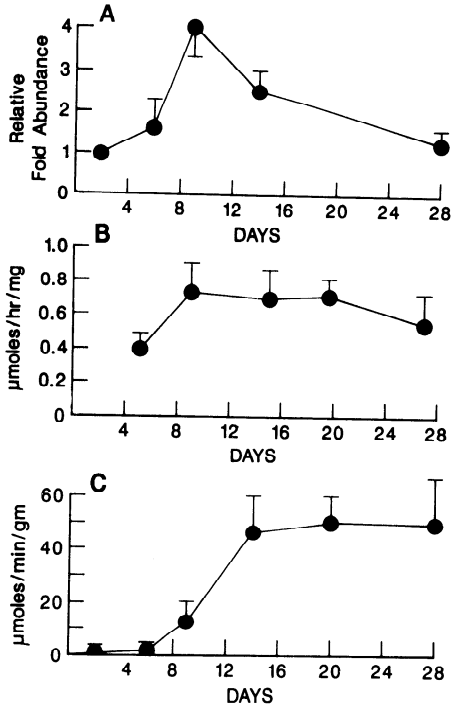

Fig. 4. Developmental profile of Caco-2 cells in culture. Alterations in expression of α_1-subunit isoform mRNA levels (*A*), Na^+-K^+-ATPase enzymatic activity (*B*), and sucrase activity (*C*) with time in culture. Data are presented as means ± SE of 3 separate experiments.

Na^+-K^+-ATPase mRNA abundance i.e., peaked 9 days after plating and remained at a plateau for 28 days. These cells also differentiate after plating (20) as shown in Fig. 4*C*. Sucrase activity, a measure of intestinal cell maturation, is lowest at plating and is maximal at ~12 days after plating.

Hormonal regulation of Na^+-K^+-ATPase gene expression in Caco-2 cells. Na^+-K^+-ATPase gene expression is known to be responsive to hormonal regulation in intestine (6, 30), as well as in numerous other tissues (13). Therefore, it was of interest to determine whether the α_1- and β_1-subunits were modulated by hormones in the Caco-2 cell line.

Caco-2 cells were treated separately with dexamethasone, aldosterone, T_3, and glucagon, and the results are illustrated in Table 3. Compared with untreated cells, T_3 stimulated both α_1- and β_1-gene transcript levels. Slot-blot analysis and quantitation by densitometry (Table 3) revealed that α_1-mRNA was increased 3-fold, and β_1 expression was increased 14-fold. T_3 increased Na^+-K^+-ATPase enzymatic activity 2-fold. Dexamethasone increased α_1- and β_1-mRNA abundance 2-fold, but only the former achieved statistical significance. Dexamethasone increased enzymatic activity by only 50%. Aldosterone and glucagon treatment did not significantly alter mRNA or enzymatic activity.

Because the most consistent effect was seen with T_3, and because T_3 is known to upregulate Na^+-K^+-ATPase in nonintestinal tissues, the responsiveness of Caco-2 cells to T_3 was studied in greater detail. Dose-response studies revealed that enzymatic activity and α_1- and β_1-subunit mRNA were significantly increased and maximal

Table 3. *Effect of various hormones on Na^+-K^+-ATPase α_1- and β_1-mRNA abundance and Na^+-K^+-ATPase activity in Caco-2 cells*

	mRNA Abundance		Enzymatic Activity
	α_1	β_1	
Control	1	1	100
Dexamethasone	2.43±0.29*	2.93±0.67	153±41.7
Aldosterone	1.56±0.42	4.82±2.29	136±48.5
Triiodothyronine	3.42±1.17†	14.52±3.63†	206.3±49.1‡
Glucagon	1.31±0.40	1.61±0.17	116.9±20.7

All data are means ± SE of 3 or 4 separate experiments. mRNA abundance is expressed as relative degree of abundance as determined by slot-blot analysis. A value of 1 was arbitrarily assigned to control (untreated) cells, and the various hormone-treated cells were normalized to that value. Na^+-K^+-ATPase activity is expressed as percent of control activity. Dexamethasone, aldosterone, and triiodothyronine concentrations were 50 nM and glucagon concentration was 0.5 nM. * $P < 0.01$; † $P < 0.05$; ‡ $P < 0.02$.

at T_3 concentrations of 15–50 nM (data not shown). All subsequent studies utilized 50 nM T_3. Time-course studies revealed that α_1- and β_1-subunit mRNAs were significantly increased at 24 and 48 h after addition of T_3, whereas enzymatic activity was not increased until 48 h after T_3 addition (data not shown).

The human β_1-gene was chosen for further study aimed at defining potential transcriptional mechanisms involved in the T_3 regulation of Na^+-K^+-ATPase gene expression in Caco-2 cells. Transfection of Caco-2 cells with the Hβ_1-1141CAT gene construct resulted in expression of CAT activity under basal conditions. When Caco-2 cells were treated with T_3, an approximately three- to fivefold increase in CAT activity was seen (Fig. 5). Transfection of Caco-2 cells with a gene construct containing an 814-bp deletion of the 5′ region of the β_1-gene fragment (Hβ_1-327CAT) resulted in a marked decrease in expression of basal CAT activity, and treatment with T_3 did not induce further activity.

DISCUSSION

Since little is known about Na^+-K^+-ATPase gene expression in the intestine, we sought to identify the isoforms present in the epithelium of the intestine, to determine their distribution along the villus/crypt unit and along the proximal-distal extent of the intestine, and to determine whether Na^+-K^+-ATPase gene expression is hormonally regulated.

Our data demonstrate that in the rat jejunum, ileum, and colon only the α_1-and β_1-isoform mRNAs are expressed. The only previous data relating to the intestine is that of Fuller and Verity (6) and Zemelman et al. (30), who demonstrated that only the α_1-isoform mRNA is expressed in rat colon and small intestine, respectively. They also noted expression of the β_1-subunit mRNA as well. The selective expression of the α_1-isoform in intestine is similar to that found in adult kidney (28), a tissue with considerable structural and functional similarity to the intestine.

Quantitative analysis of mRNA abundance revealed regional differences in α_1- and β_1-isoform expression with

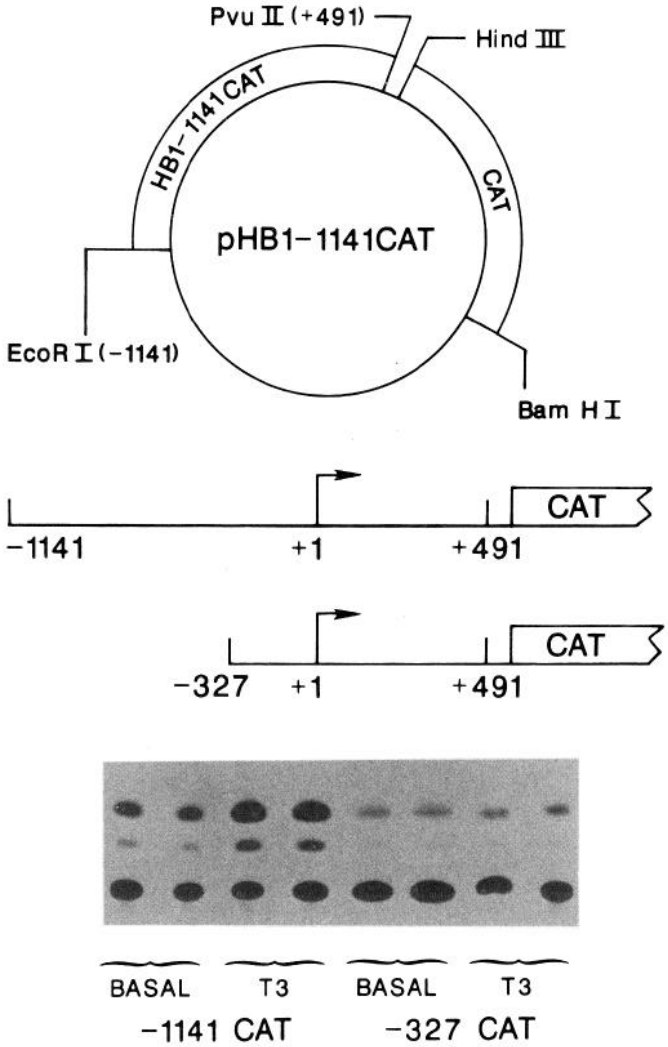

Fig. 5. Human β_1-Na^+-K^+-ATPase gene-chloramphenicol acetyltransferase (CAT) constructs and CAT expression in transfected Caco-2 cells. Constructs were prepared, transfected into Caco-2 cells, and CAT activity measured as described in MATERIALS AND METHODS. *Top*: human β_1-gene-CAT constructs. *Bottom*: autoradiogram of thin-layer chromatography plate. T_3, 3,5,3′-triiodothyronine (50 nM)-treated cells. Experiment was performed 3 times with similar results.

the greatest mRNA abundance being found in the ileal epithelium, although this did not reach statistical significance, perhaps because of the fact that this was measured only in three experiments. Interestingly, this pattern of mRNA abundance is discordant with the levels of Na^+-K^+-ATPase enzymatic activity observed in these experiments and previously (3, 16). In general, Na^+-K^+-ATPase enzymatic activity is consistently greater in jejunum and colon than in ileum (3, 16). This discordance suggests that mechanisms regulating mRNA and protein expression differ in the various portions of the intestine and that posttranscriptional mechanisms are likely to be functional in the ileum. Sucrase-isomaltase has also been shown to be regulated by posttranscriptional processes along the horizontal axis of the intestine (9). Regional differences in molecular mechanisms regulating gene expression in the intestine have also been observed with lactase (2). It is also possible that Na^+-K^+-ATPase enzymatic activity may be underestimated because of the coexpression of inhibitory activities.

A gradient in both the α_1- and β_1-subunit mRNAs also was observed in the villus-crypt unit of both the jejunum and ileum. The lowest expression was found in the crypt and increased progressively as the cells migrated to the villus tip. A similar gradient in mRNA expression has been observed for sucrase-isomaltase (26). This pattern of Na^+-K^+-ATPase gene expression in the villus-crypt unit parallels the enzymatic activity of Na^+-K^+-ATPase previously reported by us (3) and others (27).

Our data demonstrate that the administration of thyroid hormone increases the expression of both α_1- and β_1-subunit mRNAs in the rat jejunum but not in the colon. It is well known that Na^+-K^+-ATPase enzyme activity, protein abundance, and mRNA abundance in liver, kidney, skeletal, and cardiac muscle are stimulated by thyroid hormone (8, 10, 12, 22). Thyroid hormone regulation of Na^+-K^+-ATPase gene expression is a complex process involving transcriptional, posttranscriptional, translational, and posttranslational mechanisms that vary depending on the tissue (8, 10). The difference between jejunum and colon is consistent with the view that the mechanisms regulating the expression of Na^+-K^+-ATPase differ regionally in the intestine as discussed above.

Expression of Na^+-K^+-ATPase mRNAs and enzymatic activity also were studied in the human intestinal cell line Caco-2. These cells demonstrate many similarities to the rat intestine. Caco-2 cells express only the α_1- and β_1-Na^+-K^+-ATPase isoforms. A developmental profile was also seen, i.e., lowest expression 2 days after cell plating with a progressive rise to peak after ~9 days in culture. Na^+-K^+-ATPase enzymatic activity paralleled the mRNA profile.

Since Caco-2 cells exhibited similar features to the rat small intestine, we decided to use this system to determine whether Na^+-K^+-ATPase gene expression might be hormonally regulated in this model system. Our experiments demonstrated that T_3 markedly stimulated both α_1- and β_1-isoform mRNAs by ~3- and 14-fold, respectively. Interestingly, whereas T_3 augmented α_1- and β_1-mRNA abundances by 3- and 14-fold, respectively, Na^+-K^+-ATPase enzymatic activity was only increased 2-fold. A discordance between the magnitude of mRNA stimulation and enzymatic activity has also been observed in other cells such as liver (8) and suggests that posttranslational mechanisms also modulate Na^+-K^+-ATPase activity. Our observations that thyroid hormone increases α_1- and β_1-mRNA abundance in rat jejunum but not in colon suggests that, with regard to Na^+-K^+-ATPase, Caco-2 cells more closely resemble the jejunum than the colon.

Neither enzymatic activity nor mRNA abundance was stimulated by aldosterone. It is well known that the colon of various species responds to aldosterone by increasing Na^+ absorption and by increasing Na^+-K^+-ATPase activity (24). The reasons for these observed differences with our findings are unclear but might be explained by

the absence of mineralocorticoid receptors from the Caco-2 cells or by the absence of other elements of the regulatory process.

We did observe a modest twofold increase in α_1- and β_1-mRNA abundance in response to dexamethasone, although only the former reached statistical significance. Dexamethasone also resulted in a 50% increase in Na^+-K^+-ATPase enzymatic activity, which also did not reach statistical significance. As mentioned above, Fuller and Verity (6) demonstrated that dexamethasone upregulated α_1- and β_1-subunit activity in rat colon, and Zemelman et al. (30) reported that cortisone upregulated these subunits in rat small intestine. Our inability to clearly demonstrate comparable effects in Caco-2 cells could reflect the lack of appropriate steroid control mechanisms in this tissue culture line. This difference in regulation of Na^+-K^+-ATPase activity between the rat colon and small intestine Caco-2 cells may reflect intrinsic differences between the rodent intestine and these Caco-2 cells.

To gain some insight into the regulatory mechanisms by which thyroid hormone increases Na^+-K^+-ATPase mRNA abundance, we carried out DNA transfection experiments of Caco-2 cells with 5′ flanking regions of the human Na^+-K^+-ATPase β_1-gene linked to CAT. Transfected Caco-2 cells responded to T_3 treatment with a three- to fivefold increase in expression of CAT activity. These results suggest the existence of a thyroid hormone response element (TRE) in the 5′ flanking region of the β_1-gene and that regulation of the T_3-induced increase in β_1-mRNA occurs at the transcriptional level. Thyroid hormone has been shown to regulate the transcriptional activity of both the Na^+-K^+-ATPase α_1- and β_1-genes in kidney (8). We also observed that when Caco-2 cells were transfected with a plasmid containing the 5′ flanking region from which bases −1147 to −328 were deleted, basal CAT activity was markedly decreased, and the responsiveness to T_3 was lost. This suggests that the location of the TRE probably resides between nucleotides −1141 and −327. Computer analysis of the 5′ flanking sequence reveals three areas, at positions −455, −226, and −99 relative to the transcription start site, with homology to consensus sequences of known TREs (22). The identification, characterization, and functionality of the TREs in the human β_1 Na^+-K^+-ATPase gene are reported in a separate article (5a). We wish to point out, however, that our results do not exclude the possibility that posttranscriptional events may also contribute to T_3 regulation of the β_1-gene product.

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