Na,K-ATPase in Diabetic Rat Small Intestine

Changes at Protein and mRNA Levels and Role of Glucagon

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Abstract

Na,K-ATPase activity and isoform expression were measured in rat small intestinal mucosa taken from both normal and streptozocin-treated diabetic rats. Enzyme activity and abundance was 1.7–2.3-fold higher in rats diabetic for 2 wk than in controls. This was associated with 1.4–1.7-fold increases in small intestinal protein and DNA content. Ouabain inhibition curves of Na,K-ATPase were monophasic with K_is of 2.6±1.4 $\times 10^{-4}$ and 2.0±1.2 $\times 10^{-4}$ M for control and diabetic rats, respectively (NS). Northern blot analysis revealed a 2.5-fold increase in $mRNA_{\alpha 1}$ and a 3.4-fold increase in $mRNA_{\beta 1}$ in diabetic rats relative to controls. Two thirds of this increase occurred within 24 h after injection of streptozocin. Immunoblots of intestinal enzyme preparations from diabetic and control rats indicated the presence of $\alpha 1$ and $\beta 1$ subunits but not of $\alpha 2$ or $\alpha 3$. Administration of glucagon (80 μg/kg) to normal rats daily for 14–16 d increased mRNA$\alpha 1$ 3.1-fold but did not increase $mRNA_{\beta 1}$ or enzyme activity. In experimental diabetes, $\alpha 1$ and $\beta 1$ isoforms of Na,K-ATPase are coordinately upregulated at both protein and mRNA levels, an effect which appears to be partially mediated by the associated hyperglucagonemia. (*J. Clin. Invest.* 1994. 93:2725–2731.) Key words: $\alpha 1$ and $\beta 1$ isoforms • Na,K-ATPase • streptozocin • hypertrophy • small intestine

Introduction

Na,K-ATPase consists of a catalytic α subunit (111.7–112.6 kD) and a glycosylated β subunit (32–34 kD, core protein). Three isoforms of the α subunit ($\alpha 1$, $\alpha 2$, and $\alpha 3$) have been identified (1, 2). Their genes are located on different chromosomes and are expressed in a cell and tissue-specific manner (3, 4): $\alpha 1$ has been identified in virtually all tissues, $\alpha 2$ in the brain, heart, and skeletal muscle, and $\alpha 3$ in brain, heart, ciliary epithelium, and leukocytes (3, 5, 6). Rat small intestine has recently been shown to express only the $\alpha 1$ isoform (7, 8), as had previously been shown for rat kidney (2). The mRNA levels for $\alpha 1$ and $\beta 1$ in the small intestine are considerably lower at birth than in adult rats and are increased by glucocorticoids (8). Similar results were obtained for rat colon (10). Four isoforms of the β subunit have also been described in various tissues (2, 3, 5, 9, 11–15). The β subunit contributes to localization of newly synthesized α subunit molecules and also protects it from hydrolytic breakdown (14, 15).

In rats with experimental or spontaneous diabetes, maximal Na,K-ATPase activity has been reported to be decreased in heart, retina, peripheral nerve, kidney glomeruli, and skeletal muscle (16–20) but to be increased in kidney tubules and intestines (21, 22). Experimental diabetes in rats also results in increased intestinal length, perimeter, wet and dry weight, villus height, and crypt depth (23–26). There are also increases in Na-dependent sugar, amino acid, and bile acid absorption in the ileum (25, 27–32), and in the number of Na-dependent glucose carriers in ileal brush border membranes (32).

In this study of rat small intestinal mucosa, we have examined the expression of specific isoforms of Na,K-ATPase at both protein and mRNA levels and have explored the changes which occur in experimental diabetes and with administration of glucagon.

Methods

Animal preparation. Male Sprague Dawley rats were obtained from Charles River Labs (Wilmington, MA). Diabetic and age-matched control rats were housed in a light-cycled animal care facility and given ad lib access to water and regular chow. Diabetes was induced by an intraperitoneal injection of 75 mg/kg streptozocin. This dose resulted in a serum glucose level of > 300 mg/dl in 80% of rats within 24 h of injection; the remaining 20% were not used in this study. One group of diabetic rats was treated for 2–3 wk beginning 24 h after the streptozocin injection with a daily subcutaneous injection (2–8 U) of neutral protamine hagedorn (NPH) insulin. This dose was adjusted to maintain the blood sugar < 130 mg/dl. One group of normal rats was treated daily for 14–18 d with 80 μg/kg glucagon i.p. every 6 h.

Rats were sacrificed by decapitation after mild CO_2 narcosis. The small intestines were removed from the ligament of Treitz to the ileocecal junction, flushed with ice-cold 2 M NaCl and cut along the anti-mesenteric border. Small intestinal mucosae of diabetic and age matched control rats were then scraped into ice cold 2 M NaCl, washed three times with 50 mM tris 5 mM EDTA, pH 7.2, and two times with 50 mM Tris 1 mM EDTA, pH 7.2, and homogenized at 4°C in 250 mM sucrose, 30 mM histidine, and 1 mM EGTA buffer, pH 7.2. The use of hypertonic followed by hypotonic salt solutions was previously shown to loosen the mucocalyx and remove mucus from toad bladder epithelium, rendering Na,K-ATPase measurement far more accurate (33). Microsomal membranes were prepared as described by Cortas et al. (34). In some experiments sodium deoxycholate was added to the mucosal homogenate to a final concentration of 0.1% (0.48±0.06 mg DOC/mg protein).

Na,K-ATPase assays. Assays were done at 37°C in the presence of 130 mM NaCl, 20 mM KCl, 5 mM $MgCl_2$, 3 mM NaN_3, 3 mM ATP, and 30 mM Tris, pH 7.2, as previously described (33). After equalization of the ouabain concentration in all tubes, the amount of phosphate released was measured by a modification of the method of Baginski and Zak (35); 2% ammonium molybdate was added instead of 1% followed within 10 s by 2% arsenite–4% citrate–2% glacial acetic acid. Na,K-ATPase activity was expressed as μmoles P_i/mg protein/h (1 U). Assays were performed in the absence of detergent and/or in the pres-

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ence of 0.1–0.4 mg sodium dodecyl sulphate (SDS) or 1 mg sodium deoxycholate (DOC) per milligram of protein.

For ouabain inhibition curves, total homogenate or microsomal membranes were incubated for 3 h in the presence of varying concentrations of ouabain and 0.1–0.2 mg SDS/mg protein, required to stabilize the enzyme preparations. Omission of the detergent resulted in loss of up to 70% of Na,K-ATPase activity in 3 h whether or not antiproteases were added.

Alkaline phosphatase was assayed in the presence of 100 mM tris, 5 mM MgCl and 0.1 mM $ZnCl_2$, pH 10.5, as previously described (36).

DNA was determined on perchloric acid extracts of total homogenate as previously described (37, 38). RNA was measured as in Maniatis et al. (39) and protein by the method of Lowry et al. (40).

Ouabain-stimulated phosphorylation of Na,K-ATPase by orthophosphate ("back door phosphorylation"). To quantitate the number of Na pump molecules, Na,K-ATPase was phosphorylated with orthophosphate (back door phosphorylation) as previously described (41, 42). Briefly, SDS-treated microsomal membranes were incubated at room temperature for 30 min in the presence of various concentrations of phosphate (10–200 μM), SDS (0.1–0.2 mg/mg protein) with or without 4 mM ouabain. 5–20 μC_i of carrier-free ^{32}P-orthophosphate was then added (to reduce the presence of pyrophosphates and background radioactivity, the ^{32}P-orthophosphate was preincubated with bovine kidney microsomes at 37°C for 30 min followed by filtration through a 0.22-μM filter and boiling for 3 min). The reaction was quenched 30 min later with 10% BSA and 20% TCA in 0.1 mM H_3PO_4. The 12,000-g TCA pellets were washed with the same solution 3–5 times, dissolved in 300 μl of 0.1 N NaOH and 10% SDS, transferred to a specific volume of Ultima Gold scintillant fluid and counted in a Beckman scintillation counter (Beckman Instruments, Inc., Fullerton, CA). The ^{32}P bound to boiled microsomes was subtracted as background radioactivity. Ouabain-stimulated binding of phosphate (specific to Na,K-ATPase) represented the difference between binding in the presence of ouabain and in its absence. 1 mole of P_i in this assay binds to 1 mole of Na,K-ATPase. After subtracting background radioactivity, 60–85% of the signal is ouabain stimulated. Pump abundance, defined as the concentration of pumps per milligram of protein, was calculated and expressed in nanomoles of Na,K-ATPase per mg protein.

Immunoblot analysis. Partially purified enzyme was prepared according to the method of Jorgenson (43) and immunoblotted as described in Cortas et al. (34). The blots were probed with monoclonal antibodies to α1 (McK1), α2 (McB2), (provided by K. Sweadner, Harvard University, Boston, MA) and monospecific antibodies to α3, β1, and β2 obtained from upstate Biotechnology, Inc. (Lake Placid, NY). The bands were visualized either by the reduction of 5-bromo-4chloro-3-indolyl phosphate (Sigma Chemical Co., St. Louis, MO) by anti-7 mouse IgG bound alkaline phosphatase, or by labeling the antibody with radioiodinated strepavidin (^{125}I, 10^6 cpm) followed by autoradiography.

Northern blot analysis. Small intestinal mucosa was scraped and immediately frozen in liquid nitrogen. Total cellular RNA was prepared by the methods of Chirgwin et al. (44). Equal amounts of total RNA extracted from control and diabetic rats (20 μg per lane) were fractionated in a 1% agarose–6% formaldehyde 1× MOPS gel, blotted on nitrocellulose, and probed with cDNA for α1, α2, α3, β1, and β2 labeled with ^{32}P by nick translation as per Russo et al. (45). Autoradiograms were quantitated by densitometry.

Statistical analysis. Curve fitting and statistical analyses were performed by BMDPAR, a derivative-free nonlinear regression software package developed at the University of California (Los Angeles, CA) and converted for use on Dec Vax-11 by Management Science Associates (Pittsburgh, PA).

Results

Mucosal mass, protein, and DNA. 64 of 76 rats injected with streptozocin had blood glucoses above 300 mg/dl (compared to 103±4 mg/dl in vehicle-treated controls) and were used for this study. Table I shows that, after 6–8 wk of diabetes, the mean final body weight (W_f) of the streptozocin-treated rats (D3) had decreased by an average of 33% while that of age-matched controls (C3) had increased by 14%. In contrast, total protein and DNA contents in the small intestines of diabetic rats were 1.26–1.47 times greater than in controls (Table I). The length and mucosal wet weight of the small intestine also increased; at the end of 2 wk, lengths were 123±1 and 148±1 cm and mucosal wet weights were 4.9±0.5 and 7.0±0.1 g, in control and diabetic rats respectively ($P < .05$ for both differences). Results at 6–8 wk were similar. Insulin treatment of the streptozocin-treated rats prevented all these changes (Table I). There was no difference in the protein/DNA ratio between control, diabetic and insulin-treated diabetic rats.

Na,K-ATPase activity. Na,K-ATPase activity in intestinal mucosal homogenates increased 1.7–2.3-fold within 2 wk of the onset of diabetes and remained constant thereafter (Table II). This was true whether measured per milligram of protein or per milligram of DNA. The changes were of a comparable relative magnitude in detergent-treated and in nondetergent-treated homogenates. Results were similar for Na,K-ATPase activity measured in microsomal membranes (Table III). The time course for this increase is shown in Fig. 1. The first statistically significant increase in enzyme activity was seen at 6 d after streptozocin injection. Mg-ATPase and alkaline phosphatase activities in homogenates of control and diabetic rats did not differ significantly (Table III).

NPH insulin (2–8 U) administered daily for 2–3 wk to rats beginning 24 h after streptozocin injection, maintained the blood sugar below 130 mg/dl for at least two thirds of the study period and prevented the increase in Na,K-ATPase activity otherwise seen (Table II).

To determine whether the increase in Na,K-ATPase activity seen in diabetes resulted from an increase in pump abundance or from an increase in pump turnover number, the concentration of Na/K pumps in intestinal microsomal mem-

Table I. Change in Animal Weight, Intestinal Mucosal Protein, and DNA Content at 1 d, 2 wk, and 6–8 wk of Diabetes

	W_i	W_f	Protein	DNA
	g	*g*	*mg*	*mg*
C1 (n = 3)	378.3±48.2	383.5±41.1	150.5±457	7.9±1.0
C2 (n = 5)	364.4±32.8	412.4±34.7*	255.0±53.0	9.6±1.5
C3 (n = 10)	432.8±16.0	491.8±10.5*	249.5±38.6	7.5±1.3
D1 (n = 3)	373.7±54.9	344.3±56.7	209.3±82.0	7.8±0.1
D2 (n = 5)	365.4±31.7	276.6±22.7*	427.4±61.4‡	14.3±2.3‡
D3 (n = 10)	421.3±20.6	280.0±12.6*	364.0±57.7‡	10.4±1.5‡
I2 (n = 3)	388.0±10.0	431.0±23.0	193.3±36.8	6.5±1.6

Control (*C*), diabetic (*D*), and insulin-treated (*I*) rats were weighed at the time of streptozocin/vehicle injection (W_i) and just before sacrifice (W_f) at 1 d (*C1* and *D1*), 2 wk (*C2*, *D2*, and *I2*), and 6–8 wk (*C3* and *D3*). Intestines were removed from ligament of Treitz to the ileocecal valve, mucosa scraped and total mucosal protein and DNA measured. Values are means±SEM. * Different from corresponding W_i values ($P < 0.05$). ‡ Different from corresponding control values ($P < 0.01$).

Table II. Na,K-ATPase, Mg,ATPase, and Alkaline Phosphatase Activities in Intestinal Mucosal Homogenate of Control and STZ-treated Diabetic Rats

	Na,K-ATPase activity				
	(−) SDS	(+) SDS	(+) SDS	Mg,ATPase	Alk. phos.
	μM P_i/h/mg protein		*per mg DNA*	*μM P_i/h/mg protein*	
C1	7.0±0.1	—	—	8.4±0.6	—
C2	4.7±0.5	9.0±0.5	—	7.5±1.4	—
C3	4.4±0.7	9.1±1.7	134.7±15.5	7.0±0.7	33.4±6.1
D1	8.9±2.1	—	—	8.5±1.6	—
D2	10.7±0.6*	15.4±0.9*	—	8.8±0.8	—
D3	10.0±0.3*	16.3±2.3*	353.0±35.9*	6.7±0.7	41.5±12.0
I2	6.6±1.7	—	181.9±49.4	—	—

Maximal Na,K-ATPase, Mg-ATPase, and Alkaline phosphatase activities assayed in intestinal mucosal homogenate from control (*C*), diabetic (*D*), and insulin-treated (*I*) diabetic rats sacrificed at day 1 (*C1* and *D1*), 2 wk (*C2*, *D2*, and *I2*) and 6–8 wk (*C3* and *D3*) after onset of diabetes. Na,K-ATPase was measured in the presence and/or absence of detergent (see Methods). All values are means±SEM of activities. * Greater than control values ($P < 0.01$).

branes was measured by "back door" phosphorylation. As shown in Table III, diabetes caused a 2.5-fold increase in pump abundance, almost completely accounting for the increase in Na,K-ATPase activity. Na/K pump turnover did not change significantly.

To determine whether diabetes alters the affinity of small intestinal Na,K-ATPase for Na^+, mucosal homogenate enzyme activity was determined in 15-min assays at varying concentrations of Na^+ in the absence of detergent. There was no difference in the determined Kms for Na+ (14.9±1.2 mM in control rats [$n = 4$] vs 13.1±1.8 mM in diabetic rats [$n = 4$]).

Alpha isoforms of Na,K-ATPase are known to differ in their sensitivity to inhibition by ouabain (2). To determine if enzyme molecules with more than one sensitivity to inhibition by ouabain are expressed in normal or diabetic small intestinal mucosa, Na,K-ATPase activities in homogenates and microsomal membranes from control and diabetic rats were measured at different concentrations of ouabain. As shown in Fig.

Table III. Na,K-ATPase Activity, Na/K Pump Abundance and Turnover Rate in Mucosal Microsomal Membranes of Control (C3) and Diabetic (D3) Rats (6–8 wk)

	Na,K-ATPase activity		Na/K pump abundance	Na/K pump turnover	Mg,ATPase (+) DET
	(−) DET	(+) DET)			
C3	14.1±1.6	30.6±5.1	49.3±19.5	11.9±3.1	23.5±2.5
D3	23.7±2.1	68.0±12.1	125.0±31.0	9.2±1.9	24.3±2.6
	$P < 0.02$	$P < 0.02$	$P < 0.05$	(NS)	(NS)
	($n = 5$)	($n = 9$)	($n = 4$)	($n = 4$)	($n = 9$)

Values are means±SEM. ATPase activity was expressed as μmoles P_i/mg protein/h. Na/K pump abundance was expressed as picomoles of enzyme/mg protein and turnover in nanomoles P_i/min. *DET*, detergent (see Methods).

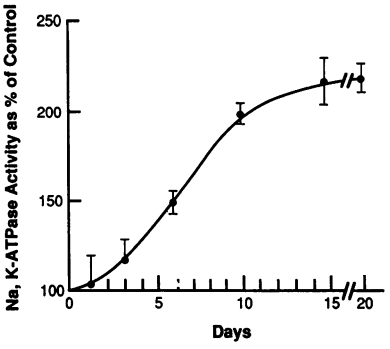

Figure 1. Time course of change in small intestinal Na,K-ATPase activity with diabetes. Enzyme activity was measured in intestinal mucosal homogenates obtained from rats with different durations of diabetes and age-matched controls. Assays were performed in the presence of SDS. The proportional (%) increase in activity in diabetes is shown as a function of duration. ($P < 0.01$ as of day 6).

2, monophasic ouabain inhibition curves were obtained for rat mucosal microsomal membranes with equivalent apparent K_is of $2.6\pm1.4 \times 10^{-4}$ M and $2.0\pm1.2 \times 10^{-4}$ M (NS) for control and diabetic rats respectively. Comparable curves and K_is were obtained with mucosal homogenates (data not shown).

Immunoblots. Immunoblots with anti-α1, α2, and α3 monospecific antibodies were initially performed on protein from mucosal homogenates and microsomal membranes from diabetic and control rats. The signal to noise ratio was too low for interpretation. We therefore partially purified the Na,K-ATPase in microsomal membranes by SDS treatment followed by centrifugation through glycerol gradient (34). These preparations showed sevenfold (120±19 μm) and 10-fold (94±9.5 μm) increases in enzyme specific activity for diabetic and control rats, respectively. SDS-PAGE analyses (34, 46) of these preparations demonstrated enrichment of the 92.5-kD band representing the α subunit(s) of Na,K-ATPase, as compared with untreated microsomal membranes (Fig. 3). The identity of this band was confirmed by immunoblot analysis. As shown in Fig. 4, α1 but not α2 or α3 were detected, as was also β1.

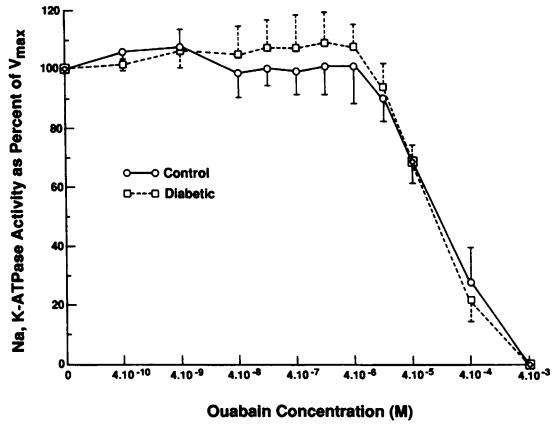

Figure 2. Na,K-ATPase activity in intestinal mucosal microsomal membranes isolated from diabetic (6–8 wk) and age-matched control rats were assayed with different concentrations of ouabain. Membranes were treated for 30 min with SDS (1 mg protein/0.3–0.5 mg SDS), diluted 15–20×, and incubated for 3 h with ouabain before assay. Na,K-ATPase activities are expressed as percent of maximum, V_{max}. (--- □ ---) Diabetic microsomes; (— ○ —) control microsomes. Each point is the mean±SEM of four experiments.

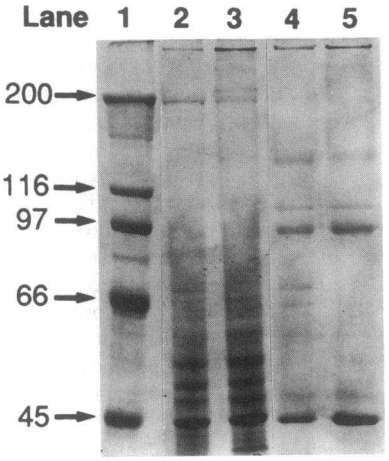

Figure 3. SDS-PAGE of partially purified Na,K-ATPase from intestinal mucosa of control and diabetic rats. The gel was stained with Coomassie blue. (Lane *1*) High molecular weight standards from top to bottom (all in kD): Myosin 200, β-galactosidase 116, phosphorylase b 92, bovine serum albumin 66, ovalbumin 45. (Lane *2*) 60 μg of intestinal mucosal microsomal membrane protein from control rats (Na,K-ATPase activity = 14 U). (Lane *3*) 60 μg of intestinal mucosal microsomal membrane protein from diabetic rats (Na,K-ATPase activity = 23 U). (Lane *4*) 30 μg of partially purified Na,K-ATPase protein from control rat intestinal mucosa (Na,K-ATPase activity = 105 U). (Lane *5*) 30 μg of partially purified Na,K-ATPase protein from diabetic rat intestinal mucosa (Na,K-ATPase activity = 150 U).

The sensitivity of the antibody probes for α2 and α3 was checked on immunoblots containing different amounts of partially purified brain (α1, α2, and α3), kidney (α1) and intestinal mucosa (α1) Na,K-ATPase preparations. In the brain, α1, α2, and α3 are equal in abundance. The minimum amounts of Na,K-ATPase required for detection of α1, α2, and α3 in the brain and α1 in intestinal mucosa were equivalent and ranged from 0.035–0.045 U of Na,K-ATPase activity. Anti-α2 and anti-α3 antibodies did not cross react with the α1 subunit in the kidney preparation. To avoid anomalous mobility of the bands on SDS-PAGE (34), we could maximally load 10 U of partially purified small intestinal Na,K-ATPase for immunoblot analysis. α1 was clearly detected but not α2. Hence α2, if present at all, would be < 0.5% of α1. The same was true for α3.

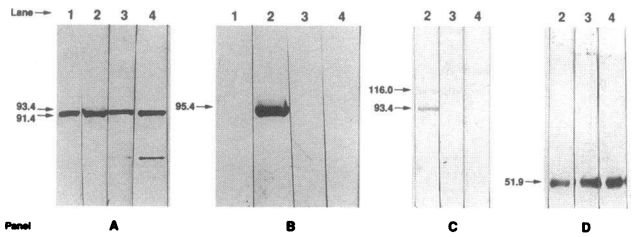

Figure 4. Partially purified Na,K-ATPase preparations from control and diabetic rat intestinal mucosa, rat brain and kidney were resolved by SDS-PAGE, electroblotted on PVDF membranes, and probed with; (*A*) McK1, a monospecific anti-α1 antibody, (*B*) McB2, a monospecific anti-α2 antibody; (*C*) a polyclonal anti-α3 antibody; (*D*) polyclonal anti-β1 antibody. Rat kidney medulla and brain Na,K-ATPase preparations were used as positive controls. In all panels; lane *1*, Na,K-ATPase from kidney medulla; lane *2*, Na,K-ATPase from brain; lane *3*, partially purified Na,K-ATPase from control intestinal mucosa; and lane *4*, partially purified Na,K-ATPase from diabetic intestinal mucosa.

Immunoblot analysis after labeling with ^{125}I-strepavidin and autoradiography also demonstrated that the increased Na,K-ATPase activity with diabetes is associated with increased abundance of α1 and β1 subunits. Enzyme abundance was measured by densitometric scanning of the autoradiograms. A constant Na,K-ATPase activity to abundance ratio was obtained for both control and diabetic preparations, indicating again similar turnover numbers.

Northern blots. To determine whether the increase in Na,K-ATPase activity and α1 and β1 abundances seen with diabetes are associated with increases in mRNA encoding these subunits, Northern blots of total RNA were probed with ^{32}P-labeled cDNA α1, α2, α3, and β1. Quantitation of the autoradiograms by densitometry revealed 2.0- and 2.1-fold increases in $mRNA_{\alpha 1}$ and $mRNA_{\beta 1}$, respectively 1 d after the onset of diabetes ($n = 3$). Maximal changes consisting of 2.5- and 3.4-fold increases in $mRNA_{\alpha 1}$ and $mRNA_{\beta 1}$, respectively, were achieved within 2 wk (Figs. 5 and 6), and maintained for at least 8 wk. $mRNA_{\alpha 2}$ and $mRNA_{\alpha 3}$ were not detected.

To determine whether the increased abundance of $mRNA_{\alpha 1}$ and $mRNA_{\beta 1}$ seen in diabetic rat intestine was due to the associated increase in serum glucagon concentration, glucagon (80 μg/kg) was administered daily to normal rats for 14–18 d. Rats were then sacrificed and their small intestinal mucosal RNA was tested with the α1 cDNA probe. There was a 3.1-fold increase in $mRNA_{\alpha 1}$ while $mRNA_{\beta 1}$ remained unchanged when compared to control rats given vehicle alone (Figs. 5 and 6). Na,K-ATPase activity in intestinal homogenates however, did not change (6.3±0.4 μm and 6.2±0.7 for glucagon-treated rats and control rats, respectively). In Figs. 5 and 6, two bands were generally detected with $cDNA_{\alpha 1}$ or $cDNA_{\beta 1}$ similar to those reported by Russo et al. (45). Young et al. (9) obtained four bands when they probed mRNA extracted from rat kidney or brain with $cDNA_{\beta 1}$, the relative intensities of the bands differed in kidney and brain. Restriction analysis revealed identical $cDNA_{\beta 1}$ in both and that the multiple mRNAs encode a single Na,K-ATPase β subunit protein. We further observed that the relative intensity of the various bands changes with the voltage and hence the total time the gel is run. In Fig. 6, lanes *1* and *3* as well as *5* and *6* are control mRNA probed with $cDNA_{\beta 1}$, the relative intensities of the upper and lower bands are different. We therefore elected to scan all bands produced by the labeled cDNA and compare total intensities.

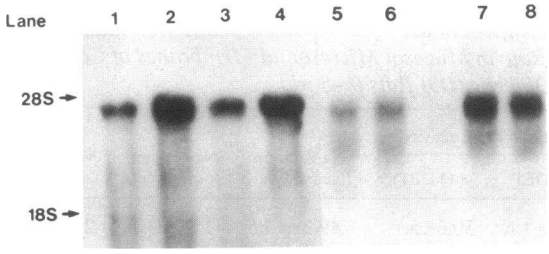

Figure 5. Detection of $mRNA_{\alpha 1}$. Autoradiograms of Northern blots of total RNA extracted from small intestinal mucosa of control and diabetic rats. Equal amounts of RNA (20 μg) were fractionated in a 1% agarose gel and transferred onto nitrocellulose membranes. The blots were hybridized with ^{32}P-dTTP labeled $cDNA_{\alpha 1}$. Lanes *1* and *3* are from control rats; lanes *2* and *4* from diabetic rats; lanes *5* and *6* from age matched controls of glucagon-treated rats; and lanes *7* and *8* from rats treated with glucagon for 2 wk.

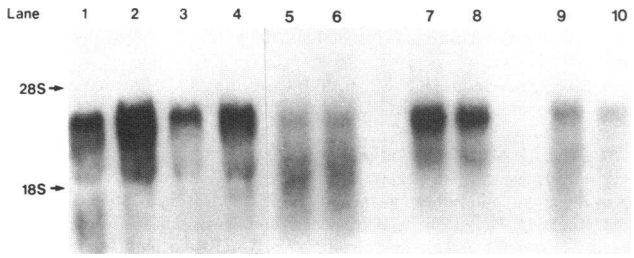

Figure 6. Detection of $mRNA_{\beta 1}$. Autoradiograms of Northern blots of total RNA extracted from small intestinal mucosa of control and diabetic rats. Equal amounts of RNA (20 μg) were fractionated in a 1% agarose gel and transferred onto nitrocellulose membranes. The blots were hybridized with ^{32}P-dTTP labeled $cDNA_{\beta 1}$. Lanes *1* and *3* are from control rats; lanes *2* and *4* from diabetic rats; lanes *5* and *6* from age matched controls of glucagon-treated rats; lanes *7* and *8* from diabetic rats; and lanes *9* and *10* from rats treated with glucagon for 2 wk.

Discussion

Streptozocin-induced diabetes in the rat results in increases in small intestinal mucosal mass, protein and DNA contents, implying an increase in the total number of intestinal epithelial cells. These became maximal within 2 wk and remained essentially constant thereafter. These findings are in agreement with histologic studies showing significant intestinal mucosal hypertrophy and hyperplasia in rats with diabetes (23–26). This hyperplasia is also seen in renal tubules (21, 47) but not in a number of other organs tested.

Similarly, increases in intestinal mucosal Na,K-ATPase activity and abundance were maximal within 2 wk. This change in enzyme activity was preceded by coordinate increases in mRNA encoding the $\alpha 1$ and $\beta 1$ subunits of Na,K-ATPase. The increase in enzyme activity was detectable by the third day of diabetes and became statistically significant by the sixth day. An increase in Na,K-ATPase activity with diabetes was previously reported in renal tubules but not glomeruli (21, 47) and in rat intestinal mucosa (22) although in neither case were its time course and transcriptional regulation determined. Hence, the two tissues, (small intestinal mucosa and renal tubule) whose mass increased with diabetes were also those in which the specific activity of Na,K-ATPase increased. The twofold increase in mRNA encoding Na,K-ATPase seen 24 h after streptozocin injection most likely preceded the onset of mucosal cell proliferation. Thymidine uptake studies are needed to confirm this.

In contrast to the intestinal mucosa and renal tubules, Na,K-ATPase activity decreased with diabetes in the heart (17), neural tissues (19, 20), and skeletal muscle (16). These decreases were seen in the absence of significant changes in tissue mass, protein and DNA contents.

Three isoforms ($\alpha 1$, $\alpha 2$, and $\alpha 3$) of the catalytic subunit of Na,K-ATPase have been detected in adult rat heart and brain but only one isoform ($\alpha 1$) in adult rat kidney (2, 34); as in the latter tissue, only the $\alpha 1$ isoform, has been detected in small intestinal mucosa (7, 8). The present study shows this to be true for both diabetic and control small intestinal mucosa as evidenced by: (*a*) monophasic ouabain inhibition curves, (*b*) immunoblot analysis of the partially purified enzyme, and (*c*) Northern blot analysis of total intestinal mucosal RNA. At functional, protein, and mRNA levels, therefore, evidence was obtained for the presence of $\alpha 1$ but not $\alpha 2$ or $\alpha 3$ subunits of Na,K-ATPase under both control and experimental diabetes conditions.

Streptozocin-induced diabetes in the rat is characterized by hyperglycemia, a decrease in serum insulin and an increase in serum immunoreactive glucagon concentrations (48, 49). It is also associated with increases in serum concentrations of mineralocorticoids and glucocorticoids, a decrease in serum concentrations of thyroid hormones, an increase in serum $[K^+]$, and a decrease in total body K^+ (50, 51). The differential regulation of Na,K-ATPase isoform(s) abundance in various tissues (myoneural vs epithelial) with diabetes is inconsistent with the known effects on Na,K-ATPase, of insulin, thyroid hormone, aldosterone and serum $[K^+]$. High concentrations of insulin increased Na,K-ATPase and/or Na^+ transport in tissues studied (52). Hypothyroidism decreases Na,K-ATPase in the gut (53). Mineralocorticoid receptors appear to be present in the colon but not in the small intestine (54, 55). The increased Na,K-ATPase activity occurring in the proximal convoluted tubules (PCT) and medullary thick ascending limb of Henle's loop (MAL) with diabetes was not prevented by adrenalectomy although that occurring in the cortical convoluted tubules (CCT) was prevented (50). In rat small intestine, a very high dose of hydrocortisone increased Na,K-ATPase by 30%, which is only a small fraction of the increase in Na,K-ATPase measured in the same study in streptozocin-induced diabetes (56). High doses of other glucocorticoids have also been shown to increase Na,K-ATPase activity in rat small intestine (9, 57, 58). As for K^+, only extreme changes incompatible with life have been shown in vitro to induce new synthesis of Na,K-ATPase (59).

Glucagon, on the other hand, was shown in the present study to cause a marked increase in $mRNA_{\alpha 1}$ but not $mRNA_{\beta 1}$. The increase in $mRNA_{\alpha 1}$ was similar in magnitude to that seen with diabetes. Measurements of intestinal enzyme activity after glucagon administration revealed no increase in enzyme activity, however. The determinants that upregulate the $\beta 1$ subunit in diabetes need to be further investigated. A prior study demonstrated that the sensitivity of rat ileal transepithelial Na^+ transport to inhibition by ouabain is decreased in experimental diabetes and that this effect could be mimicked by the in vitro addition of glucagon (60). The relation of those effects to the present findings is uncertain but could involve synthesis and basolateral membrane localization of new enzyme.

We are not aware of measurements of intestinal Na,K-ATPase in diabetic humans. In several other respects, however, intestinal changes in streptozocin-induced diabetes in the rat have been shown to mimic the human disease. This is true for the observed mucosal hypertrophy (61), for the appearance of autonomic neuropathy (62) for the enhancement of the absorptive processes for sugar and amino acids (63), and for increases in circulating levels of glucagon (48, 49).

Studies in liver epithelial cells by Koch and Leffert (64) and in 3T3 fibroblasts by Smith and Rozengurt (65) suggest a relationship between glucagon, Na,K-ATPase and cell proliferation. Trophic hormones such as glucagon, insulin and EGF were found to produce a short term (1–2 h) effect in which Na,K-ATPase activity increases in response to enhanced sodium influx and a long term (6–48 h) effect involving new protein synthesis in which Na,K-ATPase activity and abundance, as well as sugar and amino acid transports, increases. These changes were associated with ouabain-inhibitable in-

creases in DNA synthesis and cell proliferation (64–68). Increased Na,K-ATPase mRNA$_{\beta 1}$ subunit abundance preceded hepatic epithelial cell proliferation (69). A possible physiologic role of the increased Na,K-ATPase activity seen in diabetes in the small intestine, liver, and kidneys is to facilitate the also observed increases in Na^+-dependent solute transport (27).

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