Diabetes Mellitus and Glucagon Alter Ouabain-Sensitive Na⁺-K⁺-ATPase in Rat Small Intestine

RICHARD N. FEDORAK, NADIM CORTAS, AND MICHAEL FIELD

Na⁺-K⁺-ATPase provides the driving force for cellular Na⁺ transport and exists in multiple isoforms that differ in ouabain sensitivities. We report that the \( K_r \) for ouabain inhibition of glucose-evoked short-circuit current, determined in intact rat ileal mucosa mounted in Ussing chambers, is higher in streptozocin-induced chronically diabetic rats than in age-matched controls. The changes in ouabain sensitivity seen in diabetes also occurred when intact ileum of age-matched controls was incubated in vitro with \( 2.8 \times 10^{-5} \) M glucagon for at least 80 min. The effect of glucagon was blocked by cycloheximide, indicating a role for protein synthesis. This suggests that changes in ouabain sensitivity seen in diabetes are produced by glucagon, the serum concentration of which increases in diabetes.

Ouabain-dependent phosphorylation of Na⁺-K⁺-ATPase (backdoor phosphorylation) revealed a higher \( K_m \) for phosphate in intestinal basolateral membranes obtained from diabetic rats compared with age-matched controls, again confirming a decrease in ouabain sensitivity. Furthermore, the mRNA encoding the \( \alpha_1 \)-isoform was upregulated 2.6-fold in chronically diabetic intestines. This suggests that the ouabain sensitivity seen during diabetes may be due to upregulation of the \( \alpha_1 \)-isoform, known to be less sensitive to ouabain than the other isoforms. Diabetes 40:1603–10, 1991

Na⁺-K⁺-ATPase couples the hydrolysis of ATP to a flow of cations against their electrochemical gradient and is the principal mechanism responsible for establishing and maintaining low intracellular Na⁺ concentrations and high intracellular K⁺ concentrations in virtually all animal cells. The transport of Na⁺ out and K⁺ into the cell, which generates transmembrane ion gradients, regulates cell volume, resting membrane potential, Na⁺-coupled transport of organic and inorganic solutes, and electrical excitability of nerve and muscle. Purification studies showed that Na⁺-K⁺-ATPase consists of two subunits in an equimolar ratio, i.e., \( \alpha_2\beta_2 \)-tetramer, with molecular weights deduced from cDNAs of 112,000–117,000 for the large \( \alpha \) catalytic subunit and between 40,000 and 60,000 for the small \( \beta \)-glycoprotein subunit (1–3). The amino acid sequences of both subunits have been derived from the nucleotide sequences of isolated DNA fragments complimentary to the mRNA encoding these subunits. These sequences are highly conserved in several species (4–6). The \( \alpha \)-subunit contains the binding sites for ATP, Na⁺, K⁺, and cardiac glycosides (7). On the other hand, the \( \beta \)-subunit is heavily glycosylated and does not contain binding sites for ATP or ions, and its precise function is unknown (8).

The affinity of Na⁺-K⁺-ATPase for the cardiac glycoside ouabain ranges between \( 3 \times 10^{-8} \) and \( 2 \times 10^{-4} \) M among species and, to a lesser extent, within tissues of the same species (9–14). The latter variability may be due to the existence of multiple isoforms of the catalytic subunit (15,16). Biochemical studies have demonstrated the existence of at least three isoforms of the catalytic subunit, \( \alpha_1 \), \( \alpha_2 \), and \( \alpha_3 \), but only the \( \alpha_2 \) and \( \alpha_3 \) being more sensitive to ouabain inhibition than \( \alpha_1 \), (9,14,17). The kidney contains predominantly the \( \alpha_1 \)-form, whereas both \( \alpha_1 \) and \( \alpha_2 \) are found in mammalian skeletal muscle (17,18) and adipose tissue (18,19); all three are found in the brain (14). Two isoforms were first described in mammalian brain and brine shrimp with the more ouabain-resistant form having a higher affinity for Na⁺ (14,20–22). Molecular cloning techniques have identified the third isoform, \( \alpha_3 \), in rat brain (15).

This study provides evidence for a change in the kinetics of 1) ouabain inhibition of glucose-evoked short-
were examined. Furthermore, the mRNA encoding the α-subunit of Na+-K+-ATPase was upregulated in diabetic rat intestinal mucosa.

**RESEARCH DESIGN AND METHODS**

Streptozocin (STZ) was obtained from Upjohn of Canada (Don Mills, Ontario). Crystalline bovine glucagon (1.05 U/mg glucagon and 9 × 10⁻⁶ U insulin/mg; lot nos. 33038, 33099, 33273, 33303, 33422, and 258-2J-120) was graciously supplied by Lilly (Indianapolis, IN). Short-acting beef/pork insulin (4 mg/U) was obtained from Connaught (Willowdale, Ontario, Canada). [³H]leucine (150 mCi/mmol) and carrier-free orthophosphate in acid-free aqueous solution labeled with 8 mCi/ml ³²P were obtained from Amersham Canada (Oakville, Ontario). The remainder of the chemicals were reagent grade and were obtained from Sigma (St. Louis, MO).

Male Lewis rats (250–275 g, Harlan Sprague-Dawley, Indianapolis, IN) were made diabetic with a single injection of 50 mg/kg STZ administered as previously described (23). Diabetic and age-matched control rats were housed in a light-cycled animal care facility and allowed access to water and standard rat chow ad libitum (Rodent Blox, 8604-00, Wayne Pet Food, Continental Grain, Chicago, IL). Diabetes was confirmed 48 h after STZ injection by the presence of hyperglycemia >18 mM. Acutely diabetic rats were those with persistent hyperglycemia for 7 days, whereas chronically diabetic rats were those with persistent hyperglycemia for 90 days. Neither group of animals was given exogenous insulin during the study period.

**Experimental preparations.** On the day of the experiment, rats were killed by intraperitoneal injection of 320 mg pentobarbital sodium. Thirty centimeters of ileal segment were excised, rinsed with ice-cold Ringer’s solution containing 20 mM fructose and circulated by gas lift with 5% CO₂/95% O₂ (pH 7.4). The intestine was gassed with 5% CO₂/95% O₂ (pH 7.4) at 37°C. Transepithelial electrical potential difference, electrical resistance, and Isc were determined and backdoor phosphorylation or mounted in Ussing chambers for transepithelial electrical measurements. In some experiments, the intestines were incubated with glucagon and/or insulin and gassed with 5% CO₂/95% O₂ for up to 80 min before scraping to obtain mucosa for basolateral membrane preparations. Basolateral membranes were freshly prepared on the day of experiment as described by Mircheff and Wright (25). Purification of basolateral membranes was assessed by measurement of Na⁺-K⁺-ATPase activity and expressed as nanomoles inorganic phosphate produced per hour per milligram of protein as described by Kagawa et al. (26). Inorganic phosphate produced by ATP hydrolysis was determined by the method of Tausky and Schorr (27) with the modification of Radominska-Pyret et al. (28). Na⁺-K⁺-ATPase was, on average, enriched 16.4-fold in the basolateral membrane fraction (81.5 ± 2.3 nmol inorganic phosphate • h⁻¹ • mg⁻¹ protein, n = 6) compared with whole homogenates (4.98 ± 0.4 nmol inorganic phosphate • h⁻¹ • mg⁻¹ protein, n = 6). Enrichments were the same for chronically diabetic and age-matched control rats.

Ouabain-dependent backdoor phosphorylation was assessed as follows. Intestinal basolateral membrane Na⁺-K⁺-ATPase abundance was determined with phosphorylation from [³²P]orthophosphate as described by Resh (11). In this assay, ³²P in the presence of Mg and ouabain, forms an alkali-labile covalent phosphorylated intermediate of Na⁺-K⁺-ATPase. The stoichiometry is 1 mol phosphate for 1 mol enzyme. Briefly, membranes (50–100 μg protein) were incubated for 1 h at room temperature in a total volume of 100 μl of 100 mM Tris-HEPES, 5 mM MgCl₂, and 2 mM Tris-EGTA (pH 7.5) with varying concentrations of both H₂PO₄ (10–100 μM) and ouabain (0–10⁻³ M). [³²P]H₂PO₄ (50 μCi in 10 μl),
prepared by diluting carrier-free $^{32}$P into Tris-HEPES-MgCl$_2$ buffer and filtered through a Millipore GS 0.22-$\mu$m-pore filter to remove particulate material and hence reduce background radioactivity, was then added, and the incubation continued for another 30 min. These periods were sufficient to reach equilibrium (11). The reaction was then quenched by addition of 1 ml ice-cold 20% trichloroacetic acid/0.1 M H$_3$PO$_4$. The precipitate was allowed to form for 5 min on ice, centrifuged in an Eppendorf microfuge, and washed three times with trichloroacetic acid–H$_3$PO$_4$. The final pellet was dissolved in 0.5 ml of 5% sodium dodecyl sulfate by sonication and counted for radioactivity. This protocol relies on specific binding of ouabain to Na$^+$-K$^+$-ATPase, which inhibits dephosphorylation of the $[^{32}$P]phosphate enzyme complex. $[^{32}$P]phosphate incorporated into membranes in the absence of ouabain represented nonspecific binding and was subtracted from binding in the presence of ouabain (total binding) to yield Na$^+$-K$^+$-ATPase abundance. Nonspecific binding never exceeded 30% of total binding. The maximal ouabain-dependent phosphorylation of Na$^+$-K$^+$-ATPase can be calculated by Lineweaver-Burke plots; maximal phosphorylation incorporated (y-intercept) is a measurement of Na$^+$-K$^+$-ATPase abundance ($1/B_{\text{max}}$) and the slope a measurement of phosphate affinity ($K_p/B_{\text{max}}$). A rise in the slope reflects a lower affinity for ouabain (i.e., higher $K_p$ for ouabain and less ouabain sensitivity), which in turn is reflected by a lower affinity for phosphate binding (higher $K_p$ for phosphate) (11,12,19). Because during ouabain-dependent backdoor phosphorylation the affinity for phosphate binding parallels the affinity for ouabain binding, the $K_p$ for phosphate can be used to be to determine ouabain sensitivity.

mRNA studies were performed as follows. Ileal mucosal scrapings from diabetic rats and age-matched controls were immediately frozen in liquid N$_2$. Total RNA was isolated by the cesium-chloride method as in Chirgwin et al. (29), precipitated in ethanol, and dissolved in H$_2$O. RNA concentrations were determined by spectrophotometry. Total RNA was denatured by heating at 65°C for 5 min in 5 mM sodium acetate, 5 mM EDTA, 6% formaldehyde, and 50% formamide. The RNA was fractionated on a 5% sodium dodecyl sulfate by sonication and transferred to nitrocellulose paper. Replicate Northern blots were hybridized with full-length rat Na$^+$-K$^+$-ATPase $\alpha_1$, cDNA as previously described (30). The cDNA probes for $\alpha_1$ were obtained from J. B. Lingrel (Univ. of Cincinnati, OH) and reported by Shull et al. (6). The autoradiographs were scanned by Molecular Dynamics Computing laser densitometer for RNA quantification.

Results are expressed as means ± SE. Linear regression analysis of the data was performed by the computerized Sigma Plot Scientific Graph System (Jandel, Sausalito, CA).

RESULTS

EFFECTS OF DIABETES ON KINETICS OF OUABAIN INHIBITION OF GLUCOSE-EVOKED $I_{\text{sc}}$ IN INTACT INTESTINES

Intact tissue studies. To determine the sensitivity of $\alpha$-glucose-evoked $I_{\text{sc}}$ inhibition by ouabain in the intestines, ileal mucosa from chronically diabetic (90 days), acutely diabetic (7 days), or control rats was mounted in Ussing chambers and exposed for 40 min to varying concentrations of ouabain; 20 mM $\alpha$-glucose was then added to the mucosal side. The resulting increase in $I_{\text{sc}}$, which reflects glucose-stimulated Na$^+$ transport, was recorded. Ileal mucosa from chronically diabetic rats was about fivefold less sensitive to ouabain than ileal mucosa from age-matched controls with $K_s$ of ouabain of $7.2 \times 10^{-4}$ M and $1.5 \times 10^{-4}$ M, respectively (Fig. 1). However, the ouabain sensitivity of intact ileal mucosa from acutely diabetic rats did not significantly differ from that of controls with $K_s$ of $2.4 \times 10^{-4}$ and $1.5 \times 10^{-4}$, respectively.

EFFECT OF IN VITRO INSULIN AND GLUCAGON ON OUABAIN SENSITIVITY OF INTESTINAL Na$^+$-K$^+$-ATPASE

Intact tissue studies. Diabetic rats are known to have increased serum concentrations of glucagon and decreased serum concentrations of insulin. Indeed, with the method of Soybel et al. (31), our chronically diabetic rats demonstrated a significantly elevated serum glucagon level compared with age-matched controls (2951 ± 323 [n = 10] vs. 193 ± 82 [n = 8] pg/ml, respectively).

To further examine the effects of insulin and glucagon added in vitro, intact sheets of ileal mucosa from control and chronically diabetic rats were mounted in Ussing chambers, exposed to each hormone, and subsequently tested for changes in the ouabain sensitivity of the $\alpha$-glucose-evoked $I_{\text{sc}}$. The intestines were exposed to either insulin or glucagon for 80 min; ouabain ($4 \times 10^{-4}$ M) was added for the last 40 min. The ouabain concentration chosen was intermediate between the $K_s$ for ouabain of age-matched control ($1.5 \times 10^{-4}$ M) and chronically diabetic ($7.2 \times 10^{-4}$ M) rats.

Figure 2 shows the effect of glucagon on ouabain sensitivity of $\alpha$-glucose-evoked $I_{\text{sc}}$ in chronically diabetic and age-matched control ileum. Both preparations were incubated with either glucagon or diluent (as controls) for 80 min and in the presence or absence of $4 \times 10^{-4}$ M ouabain. Ileum from chronically diabetic rats again dem-
D-Glucose (20 mM) was added to mucosal reservoir after 80-min incubation. Incubation of control intact intestine with glucagon induced a less-ouabain-sensitive form of Na+-K+-ATPase in presence or absence of ouabain (4 x 10^-4 M). Both agents were added to serosal reservoir. D-Glucose (20 mM) was added to mucosal reservoir after 80-min incubation. Incubation of control intact intestine with glucagon induced a less-ouabain-sensitive form of Na+-K+-ATPase in presence or absence of ouabain (4 x 10^-4 M). Both agents were added to serosal reservoir. D-Glucose, which was added to mucosal reservoir after 80-min incubation. Tissues were incubated in presence or absence of indicated agent in incubation solution. Values are means ± SE of duplicate determinations from 6 rats. **P < 0.005 vs. nonglucagon and ouabain-incubated age-matched control ileum.

To further investigate this glucagon effect, we examined its time course. The effect of glucagon on ouabain sensitivity seen after exposure for 80 min was not seen after exposures for 20 or 40 min (data not shown). The prolonged incubation time required for the effect suggested that protein synthesis could be involved. We therefore examined the effect of cycloheximide. Cycloheximide (7 x 10^-5 M) was added with glucagon to the Ussing chamber serosal reservoir. This concentration of cycloheximide by itself did not inhibit the tsc response to D-glucose but decreased [3H]leucine incorporation into protein by 81% (data not shown). Cycloheximide abolished the effect of glucagon on the ouabain sensitivity of D-glucose-evoked ileal tsc (Fig. 3), reinforcing the notion that the glucagon effect requires protein synthesis.

Because insulin is a counterregulatory hormone for glucagon, we next examined the effect of insulin added in vitro on ouabain sensitivity. Ileum from nondiabetic rats, mounted in Ussing chambers was exposed to insulin (6.7 x 10^-6 M) alone or insulin plus glucagon (2.8 x 10^-5 M) for 80 min; ouabain (4 x 10^-4 M) was added to each in the last 40 min. The glucagon-induced change in ouabain sensitivity of D-glucose-evoked tsc was not affected by insulin (Fig. 4). Furthermore, insulin added alone at concentrations up to 10 mM did not alter baseline tsc (data not shown).

The decreased ouabain sensitivity of glucose-evoked Na^+ transport produced by glucagon is indistinguishable from similar changes in the diabetic state and could explain the latter.

**Membrane studies.** Na^+-K^+-ATPase activity assayed in enterocyte membranes by the standard phosphate release assay described by Kagawa (26) was 5.8 ± 0.5 nmol inorganic phosphate produced · h^{-1} · mg^{-1} protein in chronically diabetic rats. However, only 8–10% of total ATPase activity was ouabain inhibitable, making it impossible to construct ouabain-inhibition curves. We, therefore,
used the ouabain-dependent backdoor-phosphorylation assay as in Resh (11) to quantify enzyme units in the basolateral membranes and study further the effect of glucagon on changes in ouabain sensitivity observed in intact ileal mucosal sheets. Ouabain-insensitive intestinal basolateral membrane phosphorylation was only 30% of total phosphorylation in this assay. The maximal ouabain-dependent binding of phosphate can be calculated by Lineweaver-Burke plots: maximal phosphate incorporated (y-intercept) is a measure of Na⁺-K⁺-ATPase abundance (1/B\text{max}), and slope is a measure of enzyme affinity (K_m/B\text{max}). A rise in the slope reflects a lower affinity for ouabain (i.e., higher K_m for ouabain and less ouabain sensitivity), which in turn is reflected by a lower affinity for phosphate binding (higher K_m for phosphate) (11,12,19). Because during ouabain-dependent backdoor phosphorylation the affinity for phosphate binding parallels the affinity for ouabain binding, the K_m for phosphate can be used to determine ouabain sensitivity.

Freshly excised sheets of rat ileum, stripped of outer muscle layers, were incubated in Ringer’s solution for 80 min at 37°C in the presence or absence of 2.8 x 10⁻⁵ M glucagon. Intestinal sections were then removed from the bath and basolateral membranes prepared (see METH-ODS). These membranes were then assayed by ouabain-dependent backdoor phosphorylation in the presence of 1 x 10⁻³ M ouabain, a concentration that completely inhibits Na⁺-K⁺-ATPase, and also in the presence of 5 x 10⁻⁶ M ouabain. Basolateral membranes from chronically diabetic ileum (without exogenous glucagon incubation) increased the apparent ouabain-dependent K_m for phosphate of Na⁺-K⁺-ATPase at both ouabain concentrations by 3.9- and 3.3-fold, respectively (Fig. 5). Furthermore, the change in the K_m for phosphate in basolateral membranes is as great in acutely diabetic rats as in chronically diabetic rats (Fig. 6). In contrast, in intact sheets of ileal mucosa, alteration of ouabain sensitivity, as determined by inhibition of glucose-evoked I_sc, occurred only in chronically but not acutely diabetic animals. These changes provide further evidence that the kinetic properties of Na⁺-K⁺-ATPase are altered by diabetes. However, despite changes in ouabain sensitivity during diabetes, the abundance of Na⁺-K⁺-ATPase (B\text{max}) was similar relative to nondiabetic controls.

Addition of 7 x 10⁻⁵ M cycloheximide simultaneously with glucagon to nondiabetic age-matched control rat ileal basolateral membranes (1 x 10⁻³ M ouabain) produced a 2.4-fold (B\text{max}) and an intermediate (2.4 x 10⁻⁴ M) in chronic diabetes, and

**DISCUSSION**

Studies on intact ileum revealed that the mean apparent K_m for ouabain, as measured by glucose-evoked I_sc, was highest (7.2 x 10⁻⁴ M) in chronic diabetes, intermediate (2.4 x 10⁻⁴ M) in acute diabetes, and
FIG. 6. Lineweaver-Burke plot of ouabain-dependent phosphorylation of Na⁺-K⁺-ATPase in acutely diabetic (•; r = 0.99) and age-matched control (○; r = 0.97) rat ileal basolateral membranes at 1 × 10⁻³ M ouabain. 1/³²P, ³²P orthophosphate; 1/P0₄, 1/phosphate. Apparent Km for phosphate increased from 19.1 ± 6.2 to 65.9 ± 7.2 μM in acute diabetes (P < 0.01). Lines were drawn by linear regression; r, correlation coefficient of lines; points, means ± SE of quadruplicate determinations in 4 rats. Basolateral membranes from acutely diabetic rats were less sensitive to ouabain than controls.

FIG. 7. Effect of cycloheximide on glucagon-induced alterations of ouabain (5 × 10⁻³ M)-dependent phosphorylation of Na⁺-K⁺-ATPase in control rat ileal basolateral membranes. 1/³²P, ³²P orthophosphate. Intact sheets of ileum were preincubated for 80 min with 7 × 10⁻⁴ M cycloheximide in the presence or absence of 2.8 × 10⁻⁸ M glucagon before membranes were prepared. •, Glucagon preincubated: r = 0.98; Km for phosphate 227.2 μM. ○, Nonglucagon pre-incubated: r = 0.97; Km for phosphate 377.8 μM. Lines were drawn by linear regression; r, correlation coefficient of lines; points, means ± SE of quadruplicate determinations in 8 rats.

FIG. 8. Northern-blot analysis of mRNA from ileal mucosa of control rat intestine. An equivalent amount (20 μg) of total RNA was applied in each of 4 lanes. Lanes C1–4 represent RNA from 4 age-matched control intestines, whereas lanes D1–4 represent RNA from 4 chronically diabetic intestines. After hybridization with cDNA α₁, and washing, X-ray films were exposed to radioactive blots for 4 h. The 28s and 18s positions are marked. mRNA encoding α₁ is increased 2.6-fold in diabetes.

The change in ouabain sensitivity seen with diabetes could result from 1) a change in the relative abundance of Na⁺-K⁺-ATPase isoforms (32); 2) expression of a ouabain-resistant gene (33); or 3) a change in the enzyme microenvironment, i.e., a change in membrane lipids or cytoskeletal elements (34). Three isoforms of the catalytic subunit of Na⁺-K⁺-ATPase, α₁, α₂, and α₃, have been reported at the membrane level; they differ markedly in their sensitivities to ouabain (32). The α₁ is the least-sensitive isoform (11,14,19), and α₂ is slightly less sensitive than α₃ (32). In brine shrimp, the isoform less sensitive to ouabain has a significantly higher affinity for Na⁺ (21). In this study, Northern blots of RNA isolated from intestinal mucosa demonstrated that the mRNA encoding α₁ in the intestine, the most ouabain-insensitive isoform, is upregulated 2.6-fold in chronically diabetic ileal membranes. This upregulation of mRNA α₁ posed the question of whether the decrease in ouabain sensitivity, seen in both intact ileal mucosa and in membranes from diabetic animals, could be due to increased expression and insertion of α₁ into the basolateral membrane. Transfection showed that both α₁ and α₂ can be expressed in different tissues and lead to expected changes in ouabain sensitivity (33,35,36). Furthermore, English et al. (37) and Fallows et al. (38) reported a 1.2-kilobase mRNA, dubbed oua'6 gene, that confers ouabain resistance but is too small to express the α₁-isofrom.
Northern-blot analysis and the linear relationship seen with backdoor phosphorylation in this study indicate a predominance of one isoform ($a_1$). The relative low abundance or absence of $a_2$ and $a_3$ preclude accurate quantitation of these isoforms. The associated increase in mRNA $a_1$ and the evidence for a significant role of $a_1$ in producing ouabain insensitivity suggest that increased expression of the $a_1$-isoform in diabetic ileal membranes may be responsible for the observed change in ouabain affinity. However, this might imply that normally enough $a_2$ and/or $a_3$ is expressed that a significant change in ouabain sensitivity can be seen. Nevertheless, Giannella et al. (39) recently demonstrated that only the $a_1$-isoform of Na+-K+-ATPase is expressed specifically in the intestinal cell line CaCo2. Whether $a_2$- and/or $a_3$-isoforms are expressed in rat small intestine remains to be determined.

In this study, the ouabain sensitivity of intestinal Na+-K+-ATPase was decreased not only after induction of diabetes but also after addition of in vitro glucagon (Fig. 2–4). Hyperglucagonemia is well described in patients and animal models with diabetes mellitus. Diabetic rats in this study had significantly elevated serum glucagon levels compared with age-matched controls. Because in vitro addition of glucagon required at least 80 min for its action on ouabain sensitivity to be manifest and was prevented by coinucubation with cycloheximide, it is likely that protein synthesis is involved. Inlenfeldt (40) demonstrated a similar protein synthesis–dependent action of glucagon on Rb uptake in isolated rat hepatocytes. Although glucagon stimulates Na+-K+-ATPase (41), its effect on ouabain sensitivity has not previously been demonstrated. Insulin, which in other tissues antagonizes some of the effects of glucagon, had no effect on Na+-K+-ATPase in our study and did not prevent the alteration induced by glucagon. In comparing the results of this study to those of Lytton et al. (18) on the effects of insulin on ouabain sensitivity of Na+-K+-ATPase in isolated adipocytes, we noticed both a similarity and a difference. In general, it is clear that the effects on ouabain sensitivity of insulin described by Lytton et al. in adipocytes and diabetes described in this study are opposite in direction. Furthermore, the effects of diabetes and glucagon that we have seen were manifest both in intact enterocytes (K for ouabain) and in isolated basolateral membranes (Km for phosphate). In contrast, Lytton et al. demonstrated the insulin effects on ouabain sensitivity only in intact adipocytes and not in plasma membranes isolated from these adipocytes. Our ability to see this change in intestinal basolateral membrane assays suggests a difference in how these changes come about. Furthermore, because of our results with insulin and glucagon, insulin deficiency is probably not the explanation for the change in ouabain sensitivity we observed in diabetic rat intestine. The mechanism by which glucagon, through protein synthesis, produces these changes is not known. Glucagon is a trophic hormone of the colon and small bowel and enhances intestinal nutrient transport. Orłowski and Lingrel (44) have demonstrated that a tissue-specific pattern of expression of mRNA encoding $a_1$, $a_2$, and $a_3$ exists during ontogene-
is, whereas in other studies with short-term incubations, mRNA $a_2$ appears to be a hormonally regulated moiety (45,46).

In this study, diabetes, probably through its effect on circulating glucagon, is shown to upregulate mRNA $a_1$. The physiological role of multiple isoforms of Na+-K+-ATPase in the intestine remains to be determined. If the Na+ affinity between intestinal isoforms varies as it does in other tissues, this may provide a mechanism for upregulating or downregulating Na+-coupled transport driven by Na+-K+-ATPase.

ACKNOWLEDGMENTS

These studies were supported by grants from the Alberta Heritage Foundation for Medical Research, the Medical Research Council of Canada, the American Diabetes Association (ADA-CU-502777), and the National Institutes of Health (DK-39515-01). R.N.F. is a recipient of a Clinical Investigatorship award from the Alberta Heritage Foundation for Medical Research.

We are grateful to Drs. J. Lingrel, J. Russo, and D. Chung for providing the cDNA probes and Dr. H.I. Tager for the serum glucagon measurements. Glucagon was generously supplied by Lilly.

Portions of this work originally appeared in abstract form (Gastroenterology 90:A1412, 1986).

REFERENCES


