Adenosine Triphosphatases of Rat Pancreatic Islets

COMPARISON WITH THOSE OF RAT KIDNEY

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ABSTRACT Electrolyte fluxes are fundamental to normal endocrine pancreatic function. Adenosine triphosphatases (ATPases) are enzyme systems believed to modulate electrolyte movements across membranes in a number of cell types. This study was undertaken to measure cation-dependent ATPases of rat pancreatic islets. In addition, we compared effects of substances which influence endocrine pancreatic function upon ATPases in homogenates of islets and kidney, the latter being a tissue which would not be expected to have a stimulus-secretion response to substances which activate islets.

Both tissues were generally similar with respect to apparent Michaelis constant (ATP) of Na^+K^+ATPase, Mg^{++}ATPase, and Ca^{++}ATPase. In islets and kidney, Na^+K^+ATPase specific activity was increased when the Na:K ratio was lowered from 250:1 (175:0.7 mM) to 5:1 (100:20 mM). Inhibition of Na^+K^+ATPase at either Na:K ratio by ouabain, an activator of secretion, and enhancement of the high-ratio Na^+K^+ATPase by diphenylhydantoin, an islet secretory inhibitor, were also common to both tissues.

Because both inhibition and enhancement of Na^+K^+ATPase could be studied at the high Na:K ratio, we examined the effect of regulators of secretion upon the activity of this enzyme. Like ouabain, substances which induce or support islet secretion, glucose 16 mM or 3.3 mM, arginine 14.2 mM (with 3.3 mM glucose), or Ca^{++} 1 mM, inhibited high-ratio islet Na^+K^+ATPase. Like diphenylhydantoin, the inhibitors of insulin secretion, diazoxide 0.22 mM, or NH_4Cl 16 mM, enhanced this islet ATPase. Neither valine, which is nonsecretogenic, nor arginine without glucose, which is a weak secretagogue, had any effect upon islet Na^+K^+ATPase. We examined the effect of these substances upon other cation-dependent islet ATPases. Ca^{++} inhibited Mg^{++}ATPase, and glucose inhibited Ca^{++}ATPase. Leucine, 22.9 mM, which induces insulin secretion in the absence of glucose, suppressed islet Ca^{++}ATPase and had no effect upon high-ratio Na^+K^+ATPase.

In contrast to the observations in the islets, most substances which influence islet function had no effect on kidney ATPases, or effects which were different from those seen in islets. Except for ouabain, none of these substances influenced the three kidney ATPases in a manner similar to that seen with islets.

These findings support the hypothesis that cation-dependent ATPases are involved in specificity of islet response to substances which influence endocrine pancreatic activity.

INTRODUCTION

There is abundant evidence which supports the concept that enzyme systems modulate cellular electrolyte fluxes (1–4). Sodium-potassium-activated adenosine triphosphatase (Na^+K^+ATPase)[1] is associated with sodium and potassium transport in many tissues (5). This enzyme also requires magnesium ions for its activity, and is, in most preparations, accompanied by a magnesium-dependent adenosine triphosphatase (Mg^{++}ATPase) (1), which is not dependent upon sodium or potassium ions. A calcium-activated adenosine triphosphatase (Ca^{++}ATPase) has been described as well (3). This latter system has variable dependency upon magnesium, sodium or potassium ions (3, 6).

There have been many investigations regarding purification and physical properties of ATPases (5), and extensive studies of reaction mechanisms, membrane physiology, and drug effects (5) have been reported.

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[1] *Abbreviations used in this paper*: Ca^{++}ATPase, calcium-activated adenosine triphosphatase; DPH, diphenylhydantoin; K_m, Michaelis constant; Mg^{++}ATPase, magnesium-dependent adenosine triphosphatase; Na^+K^+ATPase, sodium-potassium-activated adenosine triphosphatase.

However, despite the fact that ion movements are essential in cellular function, relatively few studies that relate the ATPases to specialized cell function have been performed (7–11).

The present investigations were undertaken to compare the ATPases of the rat pancreatic islets with those of the more classically studied rat kidney (12). Our data indicate that certain responses of islet ATPases may be associated with cell functions characteristic of the endocrine pancreas and are distinct from those of the kidney.

METHODS

Normal male albino Sprague-Dawley rats, weighing 300–500 g, were maintained with free access to standard diet and water. Before islet or kidney collection, animals were anesthetized with intraperitoneal pentobarbital sodium, 60 mg/kg.

Islet preparation. For Na^+K^+ATPase and Mg^{++}ATPase, pancreatic islets of Langerhans were isolated in accordance with the method of Lacy and Kostianovsky (13), collected in 1–2 ml 0.25 M sucrose and 1 mM Tris-EDTA (Fisher Scientific Co., Fairlawn, N. J.), pH 7.4, and frozen at −20°C. For each experiment, 1,000 islets were used. These were collected over 2–4 days, and stored in 10 ml solution. 1,000 islets/tube for each experiment were similarly isolated and collected for Ca^{++}ATPase in 0.3 M sucrose and 10 mM Tris (Fisher Scientific Co., Fairlawn, N. J.), pH 7.4.

Kidney preparation. For Na^+K^+ATPase and Mg^{++}ATPase, frozen, whole rat kidney was thawed and washed in cold 0.25 M sucrose and 1 mM Tris-EDTA and placed on a cold stage for micro-dissection. The kidney was sliced in longitudinal sagittal section with a scalpel. Dark-red outer renal medulla was obtained by incisions along the boundaries between cortex and light gray inner medulla (14). This region was used because preliminary experiments in our laboratory, and the work of others (14), indicated the highest Na^+K^+ATPase activity is found here. Approximately 13 mg (wet weight) of outer renal medulla was obtained for subsequent homogenization. For Ca^{++}ATPase, the kidney was thawed and washed in 0.3 M sucrose and 10 mM Tris HCl. On a cold Petri dish, approximately 13 mg (wet weight) of inner cortex (14, 15) was dissected out with a scalpel and forceps, and saved in 2 ml of sucrose-Tris solution for subsequent homogenization. Inner cortex was used for Ca^{++}ATPase assay because our preliminary experiments and the work of others (15) showed that this renal region is highest in this enzyme activity.

Homogenization of tissue. For Na^+K^+ATPase and Mg^{++}-ATPase, homogenizing solution was 0.25 M sucrose in 1 mM Tris-EDTA, whereas for Ca^{++}ATPase, a solution of 0.3 M sucrose and 10 mM Tris HCl was used.

Homogenization was done manually, with the cold solutions described above, in a 2-ml Ten Broeck tissue grinder with a 0.004–0.006-inch clearance (Kontes Glass Co., Vineland, N. J.) with six or seven vigorous passes of the plunger.

Intact islets or kidney slices could be stored for subsequent assay of Na^+K^+ATPase or Mg^{++}ATPase, for periods of up to 3 mo without loss of activity. Experiments with these systems were usually performed after 1 mo of storage of intact tissue at −20°C. However, after homogenization, enzyme assay was performed within 2–7 days to avoid loss of these qualities. For Ca^{++}ATPase, it was found that intact islets could be stored at −20°C for periods up to 3 wk without loss of activity. Kidney could be stored for up to 3 mo. However, homogenization, centrifugation, and incubation had to take place within a total of 2–5 days to avoid significant loss of activity.

Centrifugation. Homogenates which were subsequently to be assayed for Na^+K^+ATPase activity were centrifuged at 35,000 *g* in 5 ml sucrose-Tris-EDTA for 35 min in a refrigerated ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif., L5-50) with a SW 50.1 rotor and 5 cm^3 cellulose nitrate tubes (Beckman Instruments, Inc., Fullerton, Calif., 305050). Supernates[2] were discarded and pellets were resuspended in cold 1 mM Tris-EDTA. For islets, 2.6 ml of this solution was used. 0.1 ml of this solution, the vol used for subsequent assay, contained tissue from 40 islets. For kidney, 2.6 ml of suspension was used to achieve 0.5 mg (wet weight) of kidney/0.1 ml sample for subsequent enzyme assay. These preparations were rapidly frozen in a test tube on dry ice in acetone and then stored at −20°C.

Homogenates which were subsequently to be assayed for Ca^{++}ATPase were initially centrifuged in sucrose-Tris HCl at 600 *g* for 10 min in a Sorvall RC2-B refrigerated centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.) with a SM 24 rotor. The pellet was resuspended in sucrose-Tris HCl solution and rehomogenized with three to four vigorous passes of the plunger. Centrifugation of this pellet at 600 *g* for 10 min was carried out. The supernate was discarded. The pellet thus obtained was then suspended in 2.6 ml 3 mM Tris HCl and quick-frozen on dry ice-acetone for storage at −20°C, to be assayed for enzyme activity within 2–5 days. The final supernate was discarded.

Enzyme assay. Assays for ATPase activity were, from this point, performed identically in islets and kidney and were carried out as follows.

Na^+K^+ATPase and Mg^{++}ATPase. Techniques used were modified for adaptation to small amounts of islet tissues. Methods were adapted from work in non-endocrine tissue by Jorgensen and Skou (16) and Lane et al. (17). At 4°C, 0.5 ml of incubation solution was added to 0.1 ml of the above tissue homogenate suspension. This incubation solution consisted of (final concentration): 0.5 mM EDTA, 21 mM glycylglycine (Sigma Chemical Co., St. Louis, Mo.), 21 mM L-histidine (Sigma Chemical Co.), and 3.6 mM $MgCl_2$. The solution was prepared with or without NaCl and KCl in the concentrations and ratios indicated in Results. 10 μl of test substance (see below) or of 3 mM Tris buffer was then added. Duplicate samples were vortexed and preincubated at 37°C (pH 7.4) for 10 min. Next, 50 μl Tris-ATP (Sigma Chemical Co.) (final concentration 3 mM) was added to all tubes. Samples were incubated in the shaker bath for 10 min at 37°C. All incubation tubes were then placed in an ice bath and the reaction was stopped with 50 μl ice-cold 50% TCA. Correction for spontaneous hydrolysis (nonenzymatic) of ATP was made with tubes which did not contain tissue. Samples were then centrifuged at 1,100 *g* for 10 min and supernates were assayed for inorganic phosphate as described below, with the method of Fiske and Subbarow (18). Na^+K^+ATPase activity was defined as the difference between the inorganic phosphate liberated in the presence, and in the absence, of sodium and potassium. Mg^{++}ATPase activity was defined as the inorganic phosphate liberated from incubates which contained Mg^{++}, but which did not contain sodium and potassium, minus that because of spontaneous (nonenzymatic) breakdown of ATP. This latter activity was found to be equal to that seen when hydrolysis of ATP from solutions which contained Mg^{++} and

[2] The cation-dependent ATPase activities which we measured were not found in these supernates nor were they measurable in 100,000 *g* pellets of these supernates.

tissue was compared to that from solutions which contained tissue but without Mg^{++}.

Ca^{++}ATPase was assayed as follows, from the method of Nakamaru et al. (6). At 4°C, incubation tubes were prepared so as to contain, at final concentrations: Tris HCl 50 mM, $CaCl_2$ 1 mM, and ouabain 0.1 mM. The test drugs or substances to be described below, when added, were mixed and pipetted into this solution. 0.1 ml of tissue suspension was then added. Tubes were vortexed and placed in a shaker bath at 37°C (pH 7.4). As with Na^+K^+ATPase and Mg^{++}-ATPase assays, appropriate controls for spontaneous ATP degradation were prepared. This was usually 10–25% of the total inorganic phosphate liberated. After a 10-min preincubation period at 37°C, 50 μl of Tris-ATP (final concentration 3 mM) was added. Incubation of tubes, in duplicate, was continued for 45 min, and then the reaction was terminated by immersion of the tubes in ice water and quickly adding 50 μl of iced 50% TCA to each tube. Ca^{++}ATPase was defined as the difference between the inorganic phosphate liberated from ATP in the presence of calcium with and without tissue. This was found to be equal to the hydrolytic activity of tissue in the presence or absence of calcium.

Phosphate was measured by the method of Fiske and Subbarow (18), the OD was read at 660 with flow-through microcuvettes in a Gilford spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). None of the added drugs or substances described below altered characteristics of the phosphate assay standard curve. In both islets and kidney, in the ranges of activity to be described for the three ATPase systems, intraassay coefficient of variation was 12% or less.

Protein content of tissue suspensions was determined by the method of Lowry et al. (19). The ATPase activity is expressed as micromoles inorganic phosphate released per milligram of protein per hour. Mean islet protein/incubation tube was 13.5 ±0.65 SE/μg for Na^+K^+ATPase and Mg^{++}ATPase and 8.6±1.0 μg for Ca^{++}ATPase. Kidney protein/incubation tube was 29.0 ±0.7 μg for Na^+K^+ATPase and Mg^{++}ATPase and 15.1±3 μg for Ca^{++}ATPase.

Additives. Various drugs and substances with known effects on endocrine pancreatic function were added to both tissues to observe their effects upon ATPase activity. Ouabain octahydrate, L-arginine HCl, sucrose, and L-leucine were purchased from Sigma Chemical Co. Diphenylhydantoin was kindly furnished by Parke-Davis & Co., Detroit, Mich. Calcium chloride, D-glucose, and ammonium chloride were purchased from Mallinckrodt, Inc., St. Louis, Mo. L-valine was purchased from Mann Laboratories, New York. Diazoxide was kindly furnished by Schering Corp., Kenilworth, N. J.

The effects of incubations in which these drugs or substances were present were compared with activity from control incubations with the paired *t* test for statistical analysis. A variety of different additives were tested in each experiment. This was done to assure that effects of tested substances could be compared with a corresponding control on several different days.

RESULTS

Kinetic studies (Fig. 1)

To compare the characteristics of the enzymes in islets and kidney, apparent Michaelis constant (K_m) (ATP) values were measured in these tissues. In addition, because Na^+K^+ATPase activity may vary at different Na^+ to K^+ ratios (20), this enzyme was studied at high (250:1) and low (5:1) Na:K ratios. Actual concentrations of sodium and potassium were, respectively, at the high ratio, 175 and 0.7 mM, and at the low ratio, 100 and 20 mM. Mg^{++} was kept constant (3.6 mM).

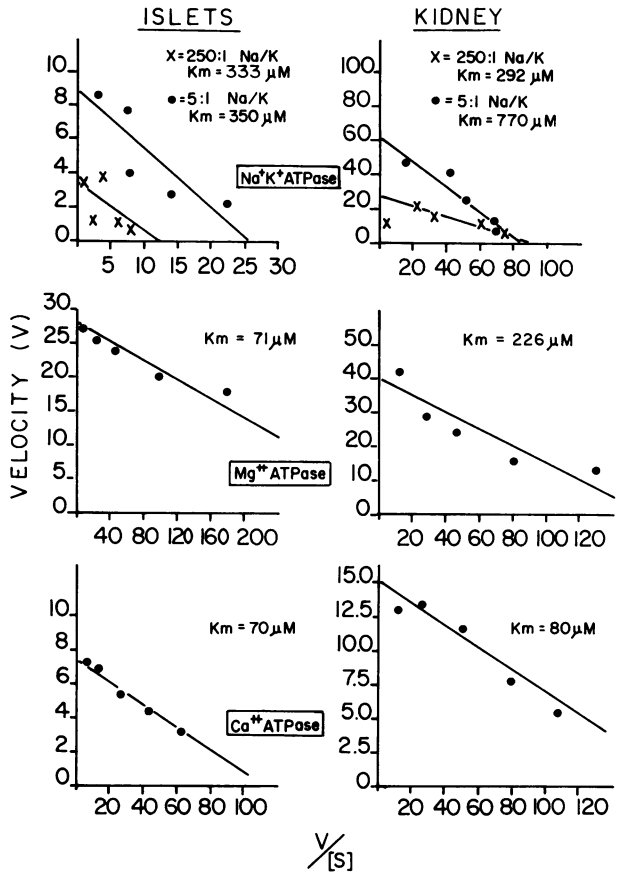

FIGURE 1 Eadie-Hofstee plots representing apparent K_m (ATP) for ATPases of islets and kidney. Note that the scaling of the ordinates and abscissae is different on several of the graphs. Each point represents the mean of three paired incubations. V = micromoles inorganic phosphate/mg protein per hour. S = substrate concentration (millimolar).

Eadie-Hofstee plots (Fig. 1) demonstrated, for Na^+K^+ATPase, similar affinity for ATP with islets at the high Na:K ratio compared to outer renal medulla. However, a lowering of the Na:K ratio did not alter K_m in islets, though this raised K_m in kidney. For Mg^{++}-ATPase, K_m in islets was one-third that of kidney. K_m for Ca^{++}ATPase was similar in both islets and renal tissue.

Specific activity. Table I describes mean control activity in the overall experiment, in which a total of more than 90,000 islets were utilized. Na^+K^+ATPase activity comprised a greater proportion of the total activity in kidney than in islets. At the 250:1 Na:K ratio, the mean percent of total $Na^+K^+Mg^{++}$ATPase activity (±SE) which was Na^+K^+ activated was 9.7±1.0 in islets and 18.5±1.6% in kidney ($P < 0.001$); whereas,

TABLE I
ATPase Specific Activity

ATPase	Islet	Kidney
	*μmol Pi/mg protein/60 min**	
Na+K+‡	2.76±0.3 (n = 34)	6.24±0.5 (n = 19)
Na+K+§	10.9±2.5 (n = 8)	40.3±4.4 (n = 9)
Mg++	25.3±1.49 (n = 42)	28.0±1.25 (n = 28)
Ca++	8.3±0.59 (n = 13)	7.7±0.54 (n = 16)

* Values given are mean±SE.
‡ With a 250:1 Na:K ratio.
§ With a 5:1 Na:K ratio.

at the 5:1 ratio, percent of total ATPase which was Na^+K^+ dependent was 24±4 in islets and 57±3% in kidney ($P < 0.001$). Moreover, a lowering of the Na:K ratio raised the percent of total ATPase which was Na^+K^+-dependent in islets and in kidney ($P < 0.001$ in both tissues). It can be seen that specific activity for Na^+K^+ATPase was less in islets than in kidney at comparable Na:K ratios ($P < 0.001$). Control specific activity, determined from ATP hydrolyzed in the presence of Na^+, K^+, and Mg^{++}, vs. hydrolysis in the absence of Na^+ and K^+, but with Mg^{++} (20), was compared with two other definitions of enzyme activity: (*a*) ATP hydrolyzed in the presence of Na^+, K^+, and Mg^{++} with and without ouabain (16), (*b*) Hydrolysis of ATP in the presence of Na^+, K^+, and Mg^{++}, vs. that seen in the absence of Na^+ and K^+, but with Mg^{++} and ouabain present (21). In 22 experiments with high-ratio Na^+K^+ATPase in islets, where the three definitions of control activity were compared, no statistical difference was found. This was also true for renal tissue.

Specific activities for Mg^{++}ATPase and Ca^{++}ATPase were quite similar in both tissues (Table I).

Effects of drugs and substances with known effects upon the pancreatic islets

Ouabain and diphenylhydantoin (DPH) (Fig. 2). Because enhancement of Na^+K^+ATPase is considered to be associated with the pharmacologic effect of DPH (22) and because, in synaptosomes, Festoff and Appel could demonstrate enhancement by DPH only at high Na:K ratios (22), we studied effects of ouabain and DPH at both the 5:1 and the 250:1 Na:K ratio. Fig. 2 demonstrates that ouabain, which induces insulin and glucagon secretion (23–27), significantly inhibited Na^+K^+ATPase in both tissues at both Na:K ratios. On the other hand, at the high Na:K ratio, DPH, an inhibitor of endocrine pancreatic function (28–36), enhanced this enzyme about 100% in islets and kidney. This enhancement was not noted at the low Na:K ratio. Neither of these drugs influenced Mg^{++}ATPase in either tissue.

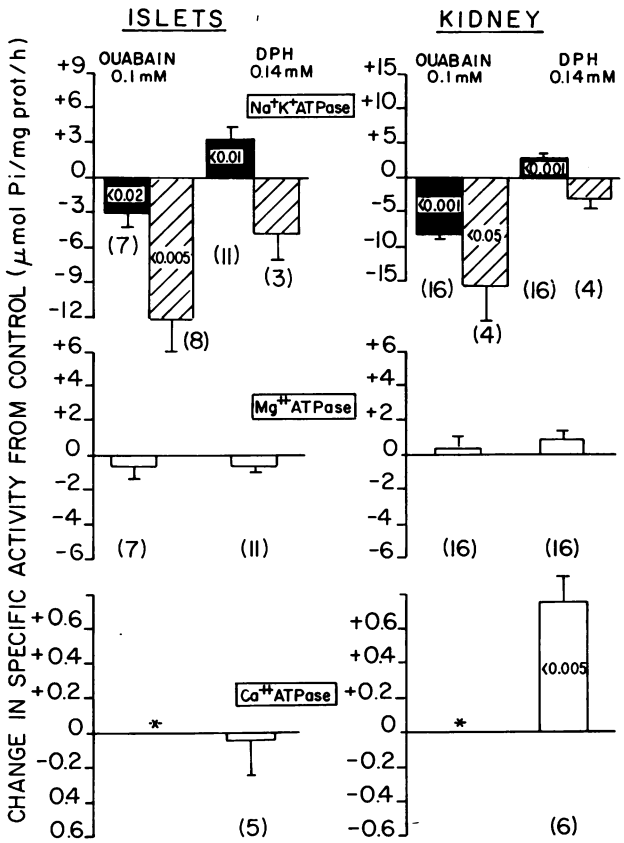

FIGURE 2 Effect of ouabain and DPH upon ATPases of islets and kidney. Changes from control are shown as mean±SEM Na^+K^+ATPase was examined in the presence of high Na:K (250:1) ratios (black columns) or of low (5:1) Na:K ratios (striped columns). Actual cation concentrations were 175:0.7 and 100:20 mM, respectively. P values indicating statistical significance are noted in response columns. Asterisk denotes that ouabain 0.1 mM was added to all Ca^{++}ATPase incubations (3, 6) so that influence of this drug on Ca^{++}ATPase could not be evaluated. Numbers in parentheses indicate the number of paired incubations.

Because ouabain was added to all tubes in the Ca^{++}-ATPase assay, to suppress any residual Na^+K^+ dependent ATPase activity (3), its effects on Ca^{++}ATPase were not measured. DPH significantly enhanced renal Ca^{++}ATPase, but did not influence this system in islets.

Because, with ouabain and DPH, both inhibition and enhancement relationships could be observed at the high Na:K ratio, the 250:1 Na:K solution (175 mM Na^+, 0.7 mM K^+) was chosen for evaluating the effects of all subsequently tested substances.

Effects of glucose and calcium (Fig. 3). In islets, glucose (16.7 mM) inhibited 100% of Na^+K^+ATPase activity as compared with the corresponding control experiments in which sp act was 3.83±0.9 SE. Glucose, 3.3 mM, partially inhibited this enzyme (28%) in islets. The difference between the inhibitory effects of

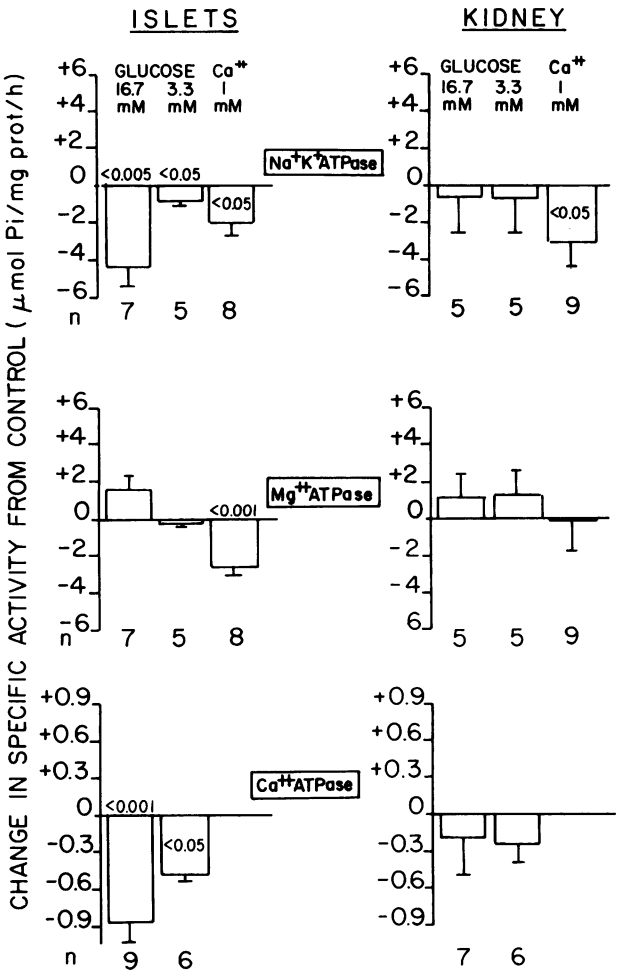

FIGURE 3 Effects of glucose and calcium upon ATPases. Changes from control are shown as mean±SEM. *P* values indicating statistical significance are noted in response columns. *n* indicates number of paired incubations.

the higher and lower glucose concentrations (4.4±0.9 vs. 0.83±0.24 μmol Pi/mg protein per h) was statistically significant ($P < 0.02$). Thus, in islets, high glucose concentrations inhibited Na^+K^+ATPase to a degree similar to that produced by ouabain. Glucose did not influence islet Mg^{++}ATPase, but at both 16.7 and 3.3 mM suppressed Ca^{++}ATPase. The degree of inhibition of Ca^{++}ATPase was not significantly greater with the high glucose concentration than it was with the lower, though trends toward a difference were seen (inhibition of 0.87±0.16 μmol Pi/mg protein per h with 16.7 mM glucose vs. 0.48±0.17 with 3.3 mM glucose). Despite prominent effects on Na^+K^+ATPase and Ca^{++}ATPase in islets, glucose had no effect on the ATPase systems in kidney. Calcium, a modulator of hormone release (37–40), inhibited both islet Na^+K^+ATPase (100%) and Mg^{++}ATPase (12%). Calcium reduced renal Na^+K^+-ATPase 51%, while having no effect upon Mg^{++}ATPase in this tissue.

Amino acids (Fig. 4). Arginine 14.2 mM, with a background glucose of 3.3 mM which results in biphasic insulin release (41), was associated with suppression of islet Na^+K^+ATPase without affecting Mg^{++}-ATPase or Ca^{++}ATPase. However, when glucose was omitted from the arginine-islet homogenate incubation tubes at a concentration which is known to give weak, monophasic insulin secretion (41), there was no influence on any of the islet ATPase activities.

In kidney, arginine suppressed Na^+K^+ATPase with or without 3.3 mM background glucose, and enhanced Mg^{++}ATPase only when glucose was present. This amino acid had no effects on Ca^{++}ATPase in islets with or without 3.3 mM glucose. On the other hand, renal Ca^{++}ATPase was enhanced by arginine in the absence of glucose. Leucine, which can produce insulin release in the absence of glucose (42), suppressed islet Ca^{++}-ATPase without significantly influencing the other two ATPases. In contrast, leucine enhanced renal Ca^{++}-ATPase and had no effect upon Na^+K^+ dependent systems.

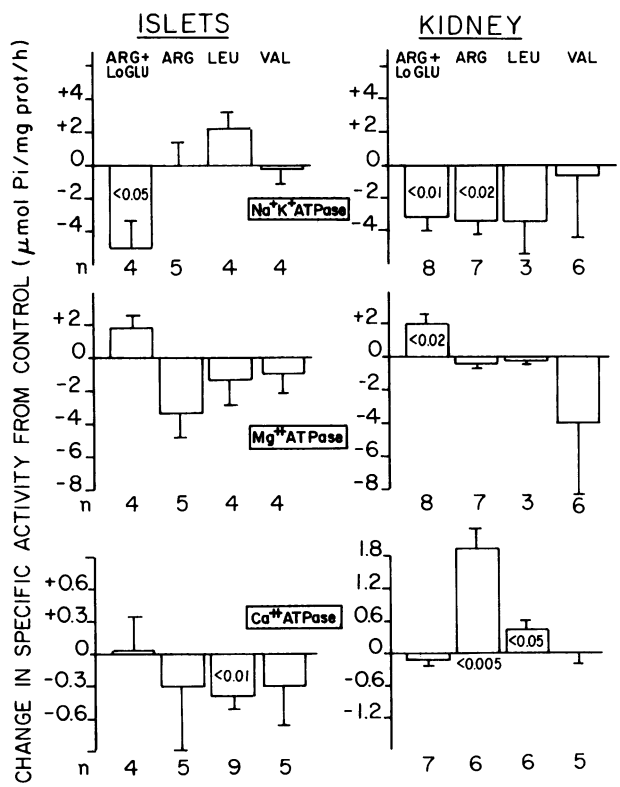

FIGURE 4 Effects of amino acids on ATPase. Changes from control are shown as mean±SEM. *P* values indicating statistical significance are noted in response columns. *n* indicates number of paired incubations. Arg = Arginine hydrochloride, 14.2 mM, Lo Glu = Glucose, 3.3 mM, Leuc = leucine 22.9 mM, Val = Valine, 14.2 mM.

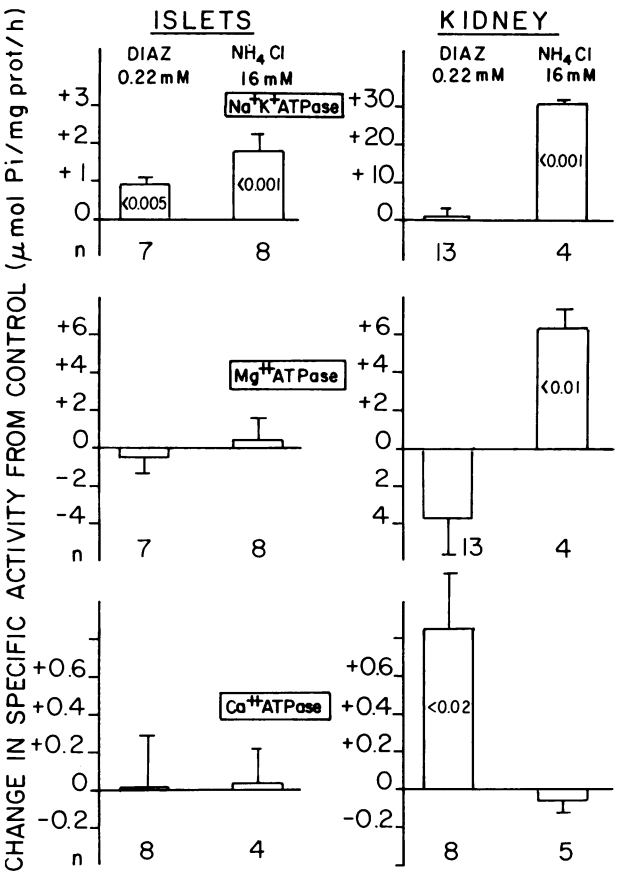

FIGURE 5 Effects of diazoxide and NH_4Cl upon ATPases of islets and kidney. Changes from control are expressed as mean±SEM. *P* values indicating statistical significance are placed in response columns. *n* indicates number of paired incubations.

Valine, which does not affect islet secretion appreciably (43), did not alter these ATPases in islets or kidney.

Diazoxide and ammonium chloride (Fig. 5). In addition to DPH, these inhibitors of islet secretion (34, 36, 44) were studied to contrast with the effects of stimulatory agents. Both diazoxide and NH_4Cl enhanced islet Na^+K^+ATPase (42% and 100%, respectively), and did not influence Mg^{++} or Ca^{++}ATPase. In kidney, responses to these agents were quite different. Diazoxide had no effect on Na^+K^+ATPase but significantly enhanced renal Ca^{++}ATPase. On the other hand, NH_4Cl enhanced both renal Na^+K^+ATPase and Mg^{++}ATPase without affecting Ca^{++}ATPase.

Table II summarizes the effect of active substances upon islet Na^+K^+ATPase. Suppression or enhancement of enzyme activity by these substances was statistically equal to 100% of the activity of paired controls. Exceptions were glucose 3.3 mM and diazoxide, which produced alterations that were statistically less than total (28% mean inhibition and 42% mean enhancement, respectively).

DISCUSSION

Previous studies of pancreatic islet function have demonstrated changes in transmembrane potential with secretagogues (45). Requisites for sodium (23), potassium (23, 46, 47) and calcium (37–39, 47) in normal endocrine pancreatic function have also been established. In these present investigations, we have measured the enzymes which are believed to modulate cationic fluxes in a variety of cell types (1, 3, 5, 11, 15). The energy for this modulation is thought to be derived from the hydrolysis of ATP.

TABLE II
Substances Which Significantly Influence High-Ratio Islet Na+K+ATPase

	Substance	(n)*	Activity: Control‡	Activity: Change induced
			μmol Pi/mg protein/60 min	
Enhancers of insulin release	Ouabain 0.1 mM	(7)	3.09±0.9	3.15±0.9 ↓
	Glucose 16.7 mM	(7)	3.83±0.9	4.42±0.2 ↓
	Glucose 3.3 mM	(5)	2.92±0.5	0.83±0.2 ↓
	Calcium 1.0 mM	(8)	3.07±0.88	1.95±0.7 ↓
	Arginine 14.2 mM + Glucose 3.3 mM	(4)	3.27±1.13	5.11±1.5 ↓
Inhibitors of insulin release	DPH 0.14 mM	(11)	2.65±0.60	3.50±1.0 ↑
	Diazoxide 0.22 mM	(7)	2.09±0.56	0.86±0.2 ↑
	NH_4Cl 16 mM	(8)	1.56±0.13	1.82±0.4 ↑

* Number of paired incubations.
‡ Paired controls which were run with each substance.

The concept that Na^+K^+ATPase is related to fundamental fluxes of these ions in islet function has had indirect support through studies of endocrine pancreatic secretion. For example, ouabain, the classic suppressor of Na^+K^+ATPase (1), enhances insulin and glucagon secretion (23–27), on the other hand, DPH, an enhancer of Na^+K^+ATPase activity (22), suppresses insulin and glucagon secretion (28–36). Ouabain increases entry of sodium into cells (48), while DPH increases the outward flux of sodium (30, 49). These cation movements are very likely the result of the influence of these drugs upon pumping action of Na^+K^+-ATPase (1, 11). These present studies demonstrate that in islets, as in other tissues, ouabain suppresses Na^+K^+ATPase and that DPH, at a high Na:K ratio, enhances this enzyme system.

The Na^+K^+ATPase system of kidney has been studied extensively (7, 14, 16, 17, 50). Because kidney does not have a known stimulus-secretion coupling reaction in response to insulinotropic substances, we have compared islet ATPases with those of kidney. We thus have here described studies which investigate whether stimulus-secretion coupling could involve suppression of Na^+K^+ATPase, as well as alterations of other cationic pump ATPases.

Our data suggests that the ATPases have similar kinetic characteristics in both islets and kidney. Apparent K_m (ATP) in islets and kidney for Na^+K^+ and Mg^{++}ATPase was in the ranges described for other tissues by Hokin (51). Similarly, our apparent K_m (ATP) of 0.08 mM for islet and kidney calcium ATPase was quite similar to that described by Formby et al. (52).

In our experiments, as in the work of others who used a variety of tissues (12, 53), larger amounts of potassium increased specific activity of Na^+K^+ATPase. The tendency of K^+ to enhance dephosphorylation of the phosphoenzyme complex of this system (2) is a likely explanation for this observation.

Ouabain suppressed Na^+K^+ATPase in all experiments, whereas DPH enhanced this enzyme activity only at high Na:K ratios. This latter finding is similar to that of Festoff and Appel (22) in neural synaptosomes. Though there are several possible explanations, no experimental data has clarified the reasons for enhancement of Na^+K^+ATPase by DPH at high Na:K ratios only. Nevertheless, we found that both inhibition and enhancement of the enzyme activity could be studied at the 250:1 (high) Na:K ratio. Under these conditions, we found that ouabain and DPH influence Na^+K^+-ATPase of both tissues; the first drug inhibits and the second enhances enzyme activity about 100%. However, in response to insulinotropic substances, islet and renal tissue differed markedly.

Glucose 16.7 mM was as potent as ouabain in suppressing islet Na^+K^+ATPase. In contrast, glucose had no effect on those renal ATPases which we measured. The concentration of glucose which is stimulatory to insulin secretion (16.7 mM) inhibited islet Na^+K^+-ATPase to a greater degree than did the nonstimulatory concentration (3.3 mM). Because threshold sensitivity to glucose is a well-described phenomenon (54), the ATPases could have a role in this sensitivity.

Glucose also inhibited islet Ca^{++}ATPase at both high and low concentrations. This enzyme system is believed to pump Ca^{++} against an electrical chemical gradient to the extracellular space (3). Because glucose induces a net uptake of islet calcium (55), the inhibition of this islet Ca^{++}ATPase would facilitate the cytoplasmic accumulation of calcium associated with insulin secretion and production of adenosine 3′,5′ cyclic monophosphate (56).

In these experiments calcium inhibited Na^+K^+ ATPase. Formby et al. (52) showed that Na^+ inhibits islet calcium ATPase. Very high concentrations of K^+, which inhibit Na^+K^+ATPase in a number of tissues (51), induce insulin secretion (57) and increase calcium uptake (58). Thus, interaction between the electrolytes and the enzymes that modulate them seems probable. Na^+ modulates Ca^{++} influx across excitable membranes in some tissues (8) and, in gastrointestinal epithelium, Na^+ entry is required for glucose entry (59), which suggests Na^+–Ca^{++} reciprocity. Microtubules of certain mammalian cells contain calcium ATPase (60) and islet microtubular function seems to require calcium fluxes (39). Calcium shifts which could influence the microtubular-microfilamentous system (61, 62) could be, in part, regulated by the enzymes described. These enzymes could also modulate a number of other calcium compartments (63).

Arginine, which with a background substimulatory glucose level results in biphasic insulin release (41), suppressed islet Na^+K^+ATPase. It had no effect on islet Na^+K^+ATPase when background glucose was omitted. In contrast, arginine suppressed Na^+K^+-ATPase in kidney with or without background glucose. Thus, arginine may nonspecifically inhibit this enzyme in a number of cells. However, the presence of small amounts of glucose appear to be a requisite for arginine-induced inhibition of islet Na^+K^+ATPase. High levels of arginine produce monophasic insulin release in vitro (41) in the absence of background glucose. Because some glucose is required for arginine-induced biphasic release, we suggest that ATPases may be related to this quality.

Leucine, which causes biphasic insulin secretion in the absence of glucose (64), inhibited islet Ca^{++}-ATPase. Because leucine causes net uptake of calcium by islets (55), it could possibly trigger insulin release independent of enzyme coupled Na^+ fluxes.

Valine, which does not cause insulin secretion (43), did not alter ATPases in islets or kidney. This again points out that amino acid activation of islet function

may be modulated through ATPases, and also suggests that the enzyme changes we noted were independent of changes in osmotic load.

In marked contrast to effects of secretagogues, the inhibitors of islet function, namely DPH, diazoxide, and ammonium chloride uniformly enhanced islet Na^+K^+ATPase. Characteristics of inhibition of insulin secretion by DPH and diazoxide have been extensively compared (34, 36). Differences in the inhibitory patterns have been emphasized and different mechanisms have been suggested with computer models (36). It is entirely possible, however, that the initial effects of these two drugs are the same, i.e., enhancement of Na^+K^+ATPase, thus leading to hyperpolarization of membranes.

Ammonium ion can substitute for K^+ (1), and presumably can participate with potassium in the dephosphorylation of the phosphoenzyme (2), which is part of the ATPase reaction. This would explain increased Na^+K^+ATPase activity with NH_4Cl.

Inhibitors of insulin secretion had no effects on islet ATPases other than Na^+K^+ATPase. However, the effects of DPH and NH_4Cl on Na^+K^+ATPase were similar in islets and kidney.

The membrane preparations used in our experiments very likely excluded many cytosolic enzyme systems (65). Thus, the influence of secretogenic substances and inhibitors upon islet ATPases could occur apart from effects upon cellular metabolism.

Lernmark et al. have recently shown that purified islet plasma membranes are rich in Na^+K^+ATPase (66). The presence of Ca^{++}ATPase has been demonstrated in microtubules of certain cells (60) and in islet mitochondria, secretory granules, and microsomes (52). These findings suggest a role for these enzyme systems in the function of specialized cell structures (67–69).

The enzyme changes we observed in islets may represent largely those of beta cells, which comprise 75–80% of rat islets (70). Possible altered function of other islet cell types which influence total enzyme activity must be considered as well.

Despite the application of similar preparatory procedures to both islets and kidney, ATPase activity in the two tissues responded quite differently to substances which influence islet secretion. Because pellets from these tissues contained all of the cation-dependent ATPase activity which we measured, the data indicates that these enzymes are involved in specificity of islet response to substances which influence endocrine pancreatic function.

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