Glomerular Na^+-K^+-ATPase Activity in Acute and Chronic Diabetes and With Aldose Reductase Inhibition

MARGO P. COHEN AND HENRY KLEPSER

Sorbitol accumulation, *myo*-inositol depletion, and reduced Na^+-K^+-ATPase activity have been identified in experimental diabetes in several tissues in which diabetic complications occur. However, a reduction in renal Na^+-K^+-ATPase activity has not been universally reported, prompting us to examine the influence of diabetes duration on the activity of this enzyme complex in isolated glomeruli. Additionally, we examined the ability of the aldose reductase inhibitor sorbinil to directly stimulate glomerular Na^+-K^+-ATPase activity in vitro, an area of interest in view of the central position that the ability of sorbinil to restore Na^+-K^+-ATPase activity in vivo occupies in the interpretive scheme that links associated changes in sorbitol, *myo*-inositol, and Na^+-K^+-ATPase to enhanced polyol-pathway activity. Glomerular Na^+-K^+-ATPase activity was significantly decreased in rats with acute (<18 days) streptozocin-induced diabetes but was significantly greater than control values in rats with more chronic (>32 days) diabetes. In vitro addition of sorbinil (1×10^{-7} M) directly stimulated Na^+-K^+-ATPase in control (0.627 ± 0.090 vs. 0.843 ± 0.098 μmol $P_i \cdot mg^{-1} \cdot min^{-1}$) but not diabetic glomeruli. These results indicate that the time of examination after induction of diabetes critically influences glomerular Na^+-K^+-ATPase activity and suggest that sorbinil, at least in normal glomerular tissue, has a membrane-associated effect that may be independent of its aldose reductase–inhibiting property. *Diabetes* 37:558–62, 1988

Results of recent studies have demonstrated a reduction in Na^+-K^+-ATPase activity in experimental diabetes in several tissues, including ocular lens, aorta, retinal pigment epithelium, and peripheral nerve (1–4). It has been proposed that the reduced Na^+-K^+-ATPase activity participates in the development of diabetic complications in these tissues, although the link between altered Na^+-K^+-ATPase and the functional and/or structural changes that characterize these complications is not entirely clear. Decreased Na^+-K^+-ATPase appears to relate to sorbitol accumulation and/or *myo*-inositol depletion, which occur on activation of the polyol pathway in the presence of hyperglycemia, because all three changes are prevented by the administration of an aldose reductase inhibitor (5–7). Cell *myo*-inositol depletion is believed to negatively influence the turnover of plasma membrane phosphoinositides, a key event in mediating signal-initiated processes, or to otherwise interfere with the integrity of the Na^+ pump, perhaps by compromising endogenous phosphatidylinositol or by a protein kinase C–related defect (2,8–11).

We have identified a similar set of abnormalities in renal glomeruli in experimental diabetes, i.e., increased sorbitol and decreased *myo*-inositol content, decreased Na^+-K^+-ATPase activity, and prevention of all three changes by treatment with the aldose reductase inhibitor sorbinil (12,13). However, some investigators have reported an increase in renal Na^+-K^+-ATPase in experimental diabetes (14–17). Because these studies differ with respect to methodology, renal preparation, and duration of diabetes, we elected to examine the effect of acute versus chronic untreated diabetes on Na^+-K^+-ATPase activity in preparations from isolated glomeruli. Additionally, we examined the effect of sorbinil in vitro on glomerular Na^+-K^+-ATPase activity. These experiments were considered of interest in view of the central position that the ability of sorbinil to restore Na^+-K^+-ATPase activity in vivo occupies in the interpretive scheme that links associated changes in sorbitol, *myo*-inositol, and Na^+-K^+-ATPase to enhanced polyol-pathway activity (11,18). That sorbinil might have a direct effect on the Na^+ pump was suggested by its lipophilicity, its influence on processes related to membrane-associated complexes such as ion transport and impulse propagation, and its ability to bind to cell membranes (19).

From the University City Science Center, Philadelphia, Pennsylvania.
Address correspondence and reprint requests to Margo P. Cohen, MD, University City Science Center, 3508 Market Street, Suite 420, Philadelphia, PA 19104.
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TABLE 1
Experimental animal data

	Control		Diabetic	
	Acute	Chronic	Acute	Chronic
Body weight (g)	272 ± 7.3	321 ± 5.4*	218 ± 9.5*†	237 ± 7.5*†‡
Kidney weight (g)	2.35 ± 0.09	2.82 ± 0.05*	2.51 ± 0.09*†	3.18 ± 0.16*†‡
Kidney-to–body-weight ratio (mg/g)	8.60 ± 0.16	8.77 ± 0.08	11.51 ± 0.08*†	13.42 ± 0.19*†‡
Glycosylated protein§ (%)	8.7 ± 0.5	8.4 ± 0.6	12.6 ± 0.3*†	15.8 ± 0.6*†‡

Acute diabetes is <18 days duration and includes corresponding age-matched control rats; $n = 4$ in each group. Chronic diabetes is >32 days' duration and includes corresponding age-matched control rats; $n = 5$ in each group.
*$P < .05$, compared with acute control.
†$P < .05$, compared with chronic control.
‡$P < .05$, compared with acute diabetic.
§Measured by Glyc-Affin (Isolab) with simultaneously determined control standard of 8.5%.

MATERIALS AND METHODS

Age-matched male white rats (Charles River, Boston, MA) were used in all experiments. Diabetes was induced by injection of 65 mg/kg body wt streptozocin (STZ) into the tail vein when animals weighed 120–150 g. Control and diabetic animals were maintained for 11–18 or 32–38 days, thus allowing the experimental diabetes to be of short-term (<18 days, designated acute) or longer-term (>32 days, designated chronic) duration. Rats were killed by cervical dislocation, and kidneys were quickly removed, placed in cold 0.85% NaCl, and processed for glomerular isolation. Each experiment was performed with glomerular lysate obtained from a control and a diabetic animal killed on the same day.

Glomeruli were isolated by sieving through a series of stainless steel meshes (20) and were hypotonically lysed in 10 mM Tris-HCl followed by repeated washing and collection by centrifugation (21). After overnight storage at −70°C, glomerular Na^+-K^+-ATPase activity was measured in thawed samples as the ouabain-inhibited inorganic phosphorus (P_i) released on incubation of the preparations with ATP. Incubations were performed in 1 ml of assay solution containing 100 mM NaCl, 10 mM KCl, 1 mM EGTA, 5 mM $MgCl_2$, 100 mM imidazole, and 3 mM Na_2-ATP, pH 7.4. To inhibit Na^+-K^+-ATPase activity, KCl was omitted and 1.0 mM ouabain was added. Where indicated, 10^{-7} M sorbinil was added to the incubations. P_i release was measured by the method of Fiske and Subbarow (22).

Results were calculated as micromoles of P_i per milligram of protein per minute. Protein was measured by the method of Bradford (23). In previous experiments, we had established that glomerular ATPase activity, both total and ouabain inhibited, showed a direct relationship with time between 0 and 10 min of incubation and with protein concentration up to 20 μg/assay, that these relationships pertained with control and diabetic preparations, that ~20% of total glomerular ATPase activity was inhibited with 1 mM ouabain, and that further inhibition did not occur with higher concentrations of ouabain in incubations with either control or diabetic samples (13). All experiments described herein were therefore conducted with two concentrations of protein (5 and 10 μg) and two periods (5 and 10 min), each performed in duplicate, to yield one data point for activity in each glomerular preparation; only experiments in which appropriate relationships with time and protein were evident were accepted.

RESULTS

All STZ-induced diabetic (STZ-D) rats were markedly hyperglycemic with mean blood glucose concentrations >18 mM and manifested typical insulin-deficient diabetes. Diabetes developed within 3 days after STZ injection and persisted through the time of killing in all injected animals. Blood glucose concentrations were elevated to a similar extent in both groups of diabetic rats (21.1 ± 1.2 and 23.1 ± 2.1 mM in acute and chronic animals, respectively), but the percent of serum protein that was glycosylated at the time of death was higher in chronic than in acute diabetes. Body weights in diabetic rats, both acute and chronic, were significantly less than those in the respective control groups. Kidney weights were also increased in diabetic rats compared with control animals; kidney-to-body weight ratios were higher in diabetic than in control rats and were significantly greater in animals with diabetes for >32 days than in those with diabetes of <18 days duration (Table 1).

Composite glomerular ATPase activity did not differ in preparations from the various experimental groups (not

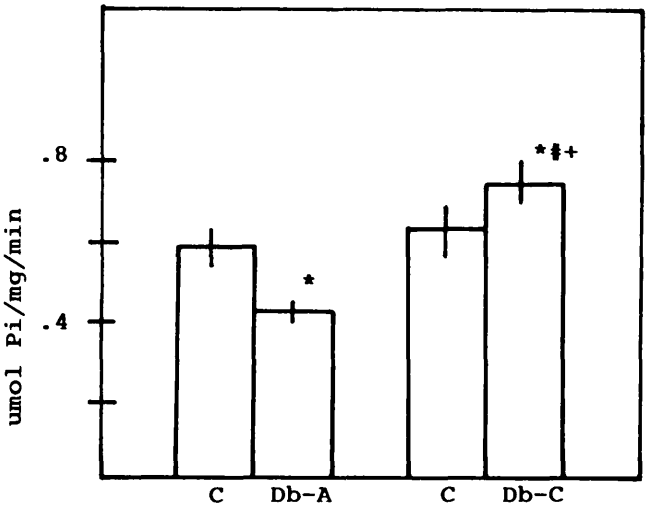

FIG. 1. Glomerular Na^+-K^+-ATPase activity in control and diabetic rats. Results represent means ± SE of individual experiments performed with preparations derived from animals described in Table 1 and with assay conditions described in text. *$P < .05$, compared with acute control (Db-A); #$P < .05$, compared with chronic control (Db-C); +$P < .05$, compared with acute diabetic.

TABLE 2
Effect of sorbinil on glomerular Na^+-K^+-ATPase activity

Experimental group	Ouabain-inhibited Na^+-K^+-ATPase (μmol P_i · mg^{-1} protein · min^{-1})	No. of experiments
Control	0.627 ± 0.090	5
Plus 10^{-7} M sorbinil	0.843 ± 0.098	5*
Acute diabetic	0.438 ± 0.028	4
Plus 10^{-7} M sorbinil	0.446 ± 0.076	4
Chronic diabetic	0.730 ± 0.089	5*
Plus 10^{-7} M sorbinil	0.764 ± 0.098	5*

Results are means ± SE. Each experiment was conducted for 2 periods with 2 concentrations of protein to yield a single data point (see text). Na^+-K^+-ATPase activity measured in the absence of KCl and the presence of 1.0 mM ouabain.
*$P < .05$, compared with control, no addition.

shown). In contrast, ouabain-inhibited glomerular ATPase activity in rats with diabetes of 11–18 days duration was significantly less than that of age-matched control preparations (Fig. 1), confirming our previous observations (13). However, ouabain-inhibited glomerular ATPase activity in preparations from animals with more chronic diabetes was significantly greater than that in the age-matched control rats, indicating that the time of examination is critical for detection of diminished glomerular Na^+-K^+-ATPase activity in experimental diabetes (Fig. 1).

The addition of 1×10^{-7} M sorbinil to incubations containing preparations from control animals had no effect on composite (total) ATPase activity but resulted in a significant stimulation of glomerular Na^+-K^+-ATPase activity. In contrast, sorbinil was without effect on either total (not shown) or ouabain-inhibitable glomerular ATPase activity in incubations performed with diabetic samples prepared from rats with acute or chronic diabetes (Table 2).

DISCUSSION

Our results confirm that reduction in glomerular Na^+-K^+-ATPase activity accompanies acute STZ-D in the rat and demonstrate that detection of this decreased activity depends on the duration of experimental diabetes. In fact, glomerular Na^+-K^+-ATPase activity is increased compared with the control when untreated diabetes is present for >3 wk. This finding may help explain an apparent dichotomy in the literature, wherein some investigators have reported an increase in renal Na^+-K^+-ATPase in experimental diabetes. Some of these studies were performed with renal tubules or used whole-tissue homogenates as an enzyme source, which would be expected to contain tubular components and, hence, to reflect stimulation of tubular ATPase activity as a result of increased Na^+ transport and/or renal hypertrophy in diabetes (14–17,24–26). Tubules and medulla contain large amounts of ATPase activity, alterations in which could obscure more subtle changes in glomerular segments in response to specific conditions (17,24,26). However, a report of increased Na^+-K^+-ATPase activity in glomeruli isolated from STZ-D rats used animals with diabetes of 4–7 wk duration (17).

The reason that glomerular Na^+-K^+-ATPase activity shifts from diminished levels early after the induction of diabetes to increased levels later in the course of untreated experimental diabetes is not clear, nor is the biological significance of these changes. However, the finding that insulin in vitro stimulates glomerular Na^+-K^+-ATPase activity (27) suggests that the early diminution reflects insulinopenia and that the renal glomerulus is a target tissue with respect to the biologic effect of insulin that influences Na^+-K^+ pump activity (28–31), although whether insulin exerts a direct effect in broken cells remains a controversial point. With untreated diabetes of longer duration, superimposed hemodynamic and other factors, e.g., stimulated Na^+-glucose transport and altered tubuloglomerular feedback, may prevail. A large increase in Na^+-K^+-ATPase of proximal convoluted tubule, fragments of which might remain attached to the isolated glomerulus, with chronic diabetes could mask less dramatic glomerular changes that might relate to the increased sorbitol and decreased *myo*-inositol known to be present in rat glomeruli after >32 days of diabetes (12).

In the context of this discussion and the findings regarding decreased Na^+-K^+-ATPase in diabetes of <18 days but not >4 wk duration, it is of interest to consider morphologic differences in glomeruli of rats with early versus chronic experimental diabetes. Extrapolating data points to the beginning of experiments that followed glomerular lesions over the course of 18 mo, Osterby and colleagues (32,33) concluded that changes of glomerular hypertrophy (increased glomerular volume, glomerular surface, total peripheral basement membrane volume, and mesangial volume) develop within days to the 1st mo after induction of diabetes and that the acute glomerular hypertrophy is arrested at an early stage and does not progress. On the other hand, Hagg and Winblad (34) reported that there is distortion of the foot processes of glomerular epithelial cells in alloxan-induced diabetic rats and that, although these changes are detectable after ~1 mo of diabetes, they become more marked (even leading to complete fusion) after 3–12 mo of diabetes (34).

The ability of sorbinil, a lipophilic spirohydantoin derivative with aldose reductase–inhibiting properties, to directly stimulate Na^+-K^+-ATPase activity in lysed preparations of isolated glomeruli is reminiscent of the effect observed with insulin (27). This effect is rapid and occurs within minutes after incubation of glomerular membranes with the hormone or with the drug. This finding supports the notion suggested by the results of previous experiments demonstrating that [^{3}H]sorbinil binds in a dose-dependent and saturable manner to crude membranes prepared from isolated rat renal glomeruli (19) and that the compound has an effect on membrane-associated complexes that is concomitant to, or independent of, its aldose reductase–inhibiting property.

Several hypotheses have been advanced to explain the ability of sorbinil in vivo to restore Na^+-K^+-ATPase activity in diabetic peripheral nerve and glomeruli. In these tissues, activation of the polyol pathway results in sorbitol accumulation and *myo*-inositol depletion, the latter perhaps due partly to inhibition of *myo*-inositol uptake or its enhanced efflux from the cell (6,35). These changes, as well as reduced Na^+-K^+-ATPase activity, are prevented by treatment of the animal with sorbinil, thus implicating increased flux of glucose through the polyol pathway and *myo*-inositol depletion and possibly involving decreased protein kinase C activity in the genesis of the decreased Na^+-K^+-ATPase activity in acute experimental diabetes (5–7,11–13,18). This postulation may require reexamination in light of the finding that sorbinil in vitro can directly stimulate Na^+-K^+-ATPase in normal glomeruli.

Garner and Spector (36) recently reported that AL 1576, an imidazolidine derivative that inhibits aldose reductase, but not sorbinil, directly stimulates purified bovine renal Na^+-K^+-ATPase. However, on the basis of dose-response curves for inhibition of fluorescein isocyanate modification of Na^+-K^+-ATPase and of elution profiles of the enzyme on fast protein liquid chromatography, these investigators concluded that sorbinil interacts with the low-affinity ATP binding site of bovine renal Na^+-K^+-ATPase. Furthermore, AL 1576, but not sorbinil, could correct the defective ATP hydrolysis induced by nonenzymatic glucosylation of pure bovine renal Na^+-K^+-ATPase, reportedly shifting the kinetics from substrate inhibition to substrate activation (36,37). These findings may be relevant to the data reported herein, which indicate that sorbinil does not exert a direct stimulatory effect on glomerular Na^+-K^+-ATPase activity in diabetic preparations. Such an effect would not be expected if the reduced activity in acute experimental diabetes related to increased (in vivo) nonenzymatic glucosylation of the enzyme protein.

In summary, our experiments demonstrate that glomerular Na^+-K^+-ATPase activity, which is decreased in acute experimental diabetes, becomes significantly increased with increasing duration of diabetes. In vitro addition of the aldose reductase inhibitor sorbinil directly stimulates Na^+-K^+-ATPase in normal, but not diabetic, glomeruli. These results indicate that the time of examination after induction of diabetes critically influences glomerular Na^+-K^+-ATPase activity and suggest that sorbinil, at least in normal glomerular tissue, has a membrane-associated effect that may be independent of its aldose reductase–inhibiting properties.

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