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Glomerular Polyol Accumulation in Diabetes and its Prevention by Oral Sorbinil

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SUMMARY

Although enhanced activity of the polyol pathway has been implicated in the pathogenesis of certain complications of diabetes, evidence that aldose reductase activity and sorbitol content are increased in the characteristic tissue site of the diabetic renal lesion has been lacking. We therefore measured polyols in glomeruli isolated from control and streptozotocin-diabetic rats, and assessed whether changes in diabetic glomeruli could be prevented by oral administration of the aldose reductase inhibitor sorbinil. Compared with control, polyol content of glomeruli isolated from diabetic rats was increased 10-fold and fourfold at 6 and 9 wk, respectively, after induction of diabetes, but was unchanged in glomeruli from rats treated with sorbinil throughout the experimental periods. In contrast, glomerular *myo*-inositol content was reduced in diabetic samples; this fall in *myo*-inositol levels was also completely prevented by sorbinil. These results establish that glomeruli contain aldose reductase activity and provide the first demonstration that glomerular polyol content increases while *myo*-inositol content decreases in diabetes and that oral sorbinil prevents these changes despite persistent hyperglycemia.

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The enhanced activity of the polyol pathway, wherein the enzyme aldose reductase converts glucose to sorbitol, that occurs in hyperglycemic states has been implicated in the pathogenesis of certain complications of diabetes. These include cataracts,[1,2] peripheral neuropathy,[3,4] and, most recently, retinopathy.[5] Whether this relationship pertains in diabetic nephropathy is entirely inferential, however, since evidence that aldose reductase activity and sorbitol content are increased in the characteristic tissue site of the diabetic renal lesion has been lacking. Aside from a report describing immunohistochemical identification of aldose reductase in renal cortical podocytes[6] and in human diabetic glomeruli,[7] and a communication indicating that renal cortex of galactosemic rats contains increased galactitol,[8] little is known about glomerular polyol metabolism and its changes in diabetes. If there is a causal link between polyol accumulation and diabetic nephropathy, then aldose reductase activity should be demonstrable in glomerular tissue and the glomerular polyol content should be elevated in diabetes. In the present study, we therefore measured polyols in glomeruli isolated from control and diabetic rats, and assessed whether changes in diabetic glomeruli could be prevented by oral administration of the aldose reductase inhibitor sorbinil. The results presented herein establish that normal glomeruli contain aldose reductase activity, and provide the first evidence that glomerular polyols increase in diabetes and that oral sorbinil prevents polyol accumulation in this tissue despite persistent hyperglycemia.

MATERIALS AND METHODS

Age-matched male white rats, separated into three groups and provided water ad libitum, were used in all experiments. Control animals received standard powdered laboratory chow. Diabetes was induced by a single injection of streptozotocin (65 mg/kg body wt) into the tail vein. One-half of the diabetic animals were given sorbinil (20 mg/kg animal weight) in the diet by mixing the appropriate amount of the aldose reductase inhibitor in powdered form into the laboratory chow. Food and sorbinil consumption were monitored daily by weighing food dishes and calculating the amount of the inhibitor ingested. The sorbinil-treated rats received the drug throughout the duration of their diabetes. Control, diabetic, and sorbinil-treated diabetic animals were maintained for 6 and 9 wk, at which time they were killed by placement in a carbon dioxide chamber. Kidneys were quickly removed from each rat, placed in cold 0.85% NaCl, and processed for glomerular isolation.

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TABLE 1
Experimental animal data

Experimental group	Duration of diabetes	Body wt (g)	mg Kidney/ g body wt
Control	—	322 ± 4 (12)	7.8 ± 0.51
Diabetic	6 wk	214 ± 22 (11)*	13.1 ± 0.60*
Diabetic and sorbinil	6 wk	178 ± 5 (12)*	13.3 ± 0.34*
Control	—	371 ± 6 (12)	7.2 ± 0.18*
Diabetic	9 wk	253 ± 5 (10)*	13.4 ± 0.47*
Diabetic and sorbinil	9 wk	207 ± 21 (11)*	14.0 ± 0.51*

Number of animals given in parentheses. Results expressed as mean ± SEM.
*Significantly different from control, $P < 0.05$.

Glomeruli were isolated by sieving through a series of stainless steel meshes.[9] For each experiment, renal cortex from 3–4 rats within the same experimental group was pooled for isolation of glomeruli. The number of glomeruli in each preparation was determined by counting three separate 0.002-ml aliquots under the light microscope at ×40 power. The glomerular preparations were well agitated immediately before removal of each aliquot to assure uniform suspension, and the total number of glomeruli in the preparation was calculated from an average of the three aliquots counted. Aliquots of the well-agitated glomerular preparations were also removed for measurement of protein by the method of Lowry et al.[10] The rest of the samples was homogenized in a solution of $ZnSo_4$ (5%), deproteinized with 0.3 N $Ba(OH)_2$, and rehomogenized as described by Varma and Kinoshita.[11] After centrifugation and lyophilization of the supernatant, samples were derivatized with BSTFA (Regisil) in pyridine, and then aliquots were subjected to gas liquid chromatography in a column standardized with fructose, α-glucose, polyol, β-glucose, and *myo*-inositol,[12] which is the order of emergence of these sugars from the column. The standards of sugars and polyol were prepared either individually or in a mixture, lyophilized, and derivatized simultaneously and in the same manner as the glomerular samples. Control and experimental samples were prepared at the same time and quantitated by integrating the area under the curve using a Numonics digitizer (Numonics Corp., Lansdale, Pennsylvania).

RESULTS

All streptozotocin-diabetic rats were markedly hyperglycemic, with mean blood glucose concentrations >20 mM/L, and manifested typical insulin-deficient diabetes. Mean blood glucose concentrations were <8 mM/L in control rats. Body weight in diabetic rats, both untreated and sorbinil-treated, was significantly less than that of control animals (Table 1). Despite loss of body weight, renal cortical mass was preserved in diabetic animals, and treatment with sorbinil had no effect on this stunted growth pattern (Table 1).

Polyol (sorbitol) was detectable and measurable in glomeruli isolated from control rats, at a concentration of 89.3 ± 6.4 (mean ± SEM) fmol/glomerulus in the 6-wk animals and 40.1 ± 3.5 fmol/glomerulus in the 9-wk animals (Table 2). The polyol content of glomeruli isolated from streptozotocin-diabetic rats was dramatically increased, reaching levels approximately 10-fold those of control at 6 wk (894.4 ± 279.0 fmol/glomerulus), and about fourfold those of control in the 9-wk animals (177.0 ± 47.2 fmol/glomerulus) (Table 2). The lower polyol content in 9-wk versus 6-wk animals is believed to be due to cell leakage as a consequence of swelling with chronic polyol accumulation.[13] Treatment with sorbinil throughout the duration of diabetes prevented polyol accumulation in glomeruli of animals maintained for either 6 wk (71.5 ± 15.3 fmol/glomerulus) or 9 wk (82.4 ± 27.0 fmol/glomerulus) (Table 2).

Increased glomerular polyols in diabetic preparations and prevention of this increase by oral sorbinil treatment were also evident when polyol content was expressed per milligram of glomerular protein (Table 2), despite the finding that the protein content of glomeruli from untreated diabetic animals was significantly greater than that in control preparations (Table 3). Thus increased protein accompanies sorbitol accumulation in individual glomeruli in diabetes. This finding is consistent with previous observations that there is an accumulation of basement membrane in glomeruli of streptozotocin-diabetic rats,[9] as well as increased synthesis and

TABLE 2
Polyol content of isolated glomeruli

Experimental group	Duration of diabetes	fmol/glom	nmol/mg Protein
Control	—	89.3 ± 6.4	0.84 ± 0.18
Diabetic	6 wk	894.4 ± 279.0*	6.39 ± 1.74*
Diabetic and sorbinil	6 wk	71.5 ± 15.25	0.48 ± 0.01
Control	—	40.1 ± 3.5	0.43 ± 0.06
Diabetic	9 wk	177.0 ± 47.2*	1.31 ± 0.39*
Diabetic and sorbinil	9 wk	82.4 ± 27.0	0.63 ± 0.12

Results given as mean ± SEM of three experiments and expressed as 10^{-15} mol of polyol per glomerulus (fmol/glom), and as nmol of polyol per mg protein in isolated glomeruli (nmol/mg). In each experiment for every experimental group, renal cortex from 3–4 rats was pooled for isolation of glomeruli.
*Significantly different from control, $P < 0.05$.

TABLE 3
Protein content of isolated glomeruli

Experimental group	Duration of diabetes	μg/glom
Control	—	0.117 ± 0.02
Diabetic	6 wk	0.136 ± 0.01*
Diabetic and sorbinil	6 wk	0.123 ± 0.02
Control	—	0.094 ± 0.08
Diabetic	9 wk	0.149 ± 0.03*
Diabetic and sorbinil	9 wk	0.123 ± 0.02

Results given as mean ± SEM of three experiments in each group, and expressed as μg of protein per glomerulus. Renal cortices from 3–4 rats were pooled for every experiment in each group as indicated in the legend to Table 2.
*$P < 0.05$ compared with controls.

decreased turnover of glomerular basement membrane collagen in diabetes.[14] It is interesting to note that protein content in glomeruli from sorbinil-treated diabetic rats was not significantly different from control, suggesting that prevention of sorbitol accumulation impacts on processes contributory to glomerular protein accumulation.

Changes in the *myo*-inositol content of glomeruli from diabetic rats were also observed. Glomerular *myo*-inositol was significantly diminished in diabetic preparations, whether expressed as pmol/glomerulus (control = 1.09 ± 0.24; 9-wk diabetic = 0.76 ± 0.19) or as nmol/mg protein (control = 11.37 ± 1.56; 9-wk diabetic = 5.46 ± 1.32). These changes were prevented by treatment with sorbinil throughout the duration of diabetes (*myo*-inositol content = 1.32 ± 0.21 pmol/glomerulus and 10.86 ± 0.88 nmol/mg protein in 9-wk, sorbinil-treated rats).

DISCUSSION

There is considerable evidence that intracellular accumulation of sorbitol, resulting from increased activity of the polyol pathway, occurs in a variety of tissues and has deleterious effects in diabetes. This pathway, the presence of which has been identified in lens,[1,2] Schwann cells of peripheral nerves,[15,16] aorta,[17] kidney papillae,[18] and pancreatic islets,[19] consists of two enzymes, aldose reductase and sorbitol dehydrogenase, that convert glucose first to the sugar alcohol sorbitol and then to the ketosugar fructose.[16] In the presence of hyperglycemia, the flux of glucose through this pathway is greatly augmented, leading to intracellular accumulation of polyol pathway products. Among the deleterious effects proposed to be associated with this accumulation are cataractogenesis, slowing of motor nerve conduction velocity, and, quite recently, retinal capillary basement membrane thickening.[5] Perhaps the most cogent evidence linking these diabetic complications to the polyol pathway is the finding that inhibition of aldose reductase activity in these tissues has a beneficial effect on their development or progression. To date, however, there has been no direct or indirect evidence supporting a relationship between diabetic nephropathy and polyol metabolism, since accumulation of polyols in glomeruli, the characteristic site of the nephropathic lesion, in diabetes has not been demonstrated. Although increased sorbitol and resultant osmotic swelling in renal papillae have been invoked as contributory to the tubular nephropathy that may be associated with uncontrolled diabetes,[16] this entity does not equate, either in anatomic site or pathologic manifestation, with diabetic glomerulosclerosis.

Using specific antibodies to aldose reductase purified from rat seminal vesicles, Ludvigson and Sorenson[6] localized aldose reductase immunohistochemically in several areas of the kidney. The most intense staining was seen in the inner medulla, while outer medulla and cortex stained faintly and inconsistently. Within the cortex, however, aldose reductase staining was found in the convoluted portions of the distal tubule and in glomerular epithelial cells (podocytes). The present results, which demonstrate measurable polyol in glomeruli isolated from normal rats, are consistent with these findings. Since our glomerular preparations are virtually devoid of tubular elements, the measured polyol should thus represent that contained in the epithelial cells. From the dramatic increase in glomerular sorbitol associated with diabetes, it is clear that polyol pathway activity in this tissue has the capacity to expand greatly in the presence of hyperglycemia. The ability of sorbinil to prevent polyol accumulation indicates that the agent enters epithelial cells and effectively inhibits aldose reductase activity despite persistent hyperglycemia. Like other aldose reductase inhibitors, sorbinil does not alter hyperglycemia; only the formation of polyol is suppressed in a dose-dependent manner.[20,21]

It is of interest to note that glomerular *myo*-inositol levels were reduced in streptozotocin-diabetic rats. Although a fall in sciatic nerve *myo*-inositol is known to occur in acute streptozotocin diabetes,[22] this finding has not previously been described in glomerular tissue. That the reduction in glomerular *myo*-inositol was prevented by sorbinil treatment is consistent with the data reported by Finegold et al., implicating the accumulation of polyol pathway intermediates as contributory to the fall in tissue *myo*-inositol content.[23] The role of reduced *myo*-inositol in diabetic glomerulopathy is currently unknown.

While the role of sorbitol accumulation in the pathogenesis of diabetic glomerulopathy remains speculative, it is interesting to note that enlargement of renal cortical podocytes, suggestive of intracellular edema, as well as abnormal cytoplasmic extensions of their cell membranes, have been described in diabetic glomerulosclerosis.[24–26] Glomerular epithelial cells participate in basement membrane synthesis, and the proteinuria occurring in diabetes has been related to the morphologic alterations in the epithelial foot processes.[27] It is also interesting to note that the renal hypertrophy associated with galactosemia, which may be analogous to diabetic hypertrophy, is diminished with aldose reductase inhibition.[28] From these considerations and the results presented herein, the possibility that glomerular polyol accumulation is pathogenetically linked to the development of diabetic nephropathy and/or influences basement membrane metabolism warrants investigation.

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