Adenosine Triphosphatase Activity in Sciatic Nerve Tissue of Streptozocin-Induced Diabetic Rats with and without High Dietary Sucrose: Effect of Aldose Reductase Inhibitors (43235)

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Abstract. **The ability of aldose reductase inhibitors to prevent the decline in neural Na^+,K^+-ATPase activity in diabetic rats has not been confirmed by all laboratories. In this study, the efficacy of two structurally different aldose reductase inhibitors was evaluated under different experimental conditions. Na^+,K^+-ATPase activity was measured in sciatic nerves from streptozocin-induced diabetic rats fed normal rodent chow or a chow supplemented with 68% sucrose. Nerve homogenates from chow-fed rats were prepared with a Dounce tissue grinder, whereas homogenates from the sucrose-fed rats were prepared with an Ultra-Turrax disperser. In the chow-fed rats, 4 weeks of untreated diabetes resulted in an increase in neural sorbitol and fructose, a decrease in myoinositol, and a 54% decline in Na^+,K^+-ATPase activity. Sorbinil administration (20 mg/kg/day) completely prevented the rise in sorbitol and fructose and the depletion of myoinositol, but did not prevent the decline in Na^+,K^+-ATPase activity. In diabetic rats fed the sucrose diet for 4, 6, and 8 weeks, the neural sorbitol and fructose levels were elevated, the myoinositol concentration declined, and the Na^+,K^+-ATPase activity was 26 to 28% below the control. Prevention or intervention treatment with sorbinil (20 mg/kg/day) or tolrestat (50 mg/kg/day) for 4 to 6 weeks prevented the alterations in sorbitol, fructose, and myoinositol, and also prevented the decline in Na^+,K^+-ATPase activity. In conclusion, prevention and intervention therapy with aldose reductase inhibitors prevented the decline in Na^+,K^+-ATPase in sciatic nerves of sucrose-fed streptozocin-diabetic rats that were homogenized with an Ultra-Turrax disperser, but not in sciatic nerves from streptozocin-diabetic rats fed normal rodent chow that were homogenized with a Dounce tissue grinder. These findings indicate that the assessment of aldose reductase inhibitor efficacy is dramatically affected by the type of nerve preparation assayed and/or the diet.** [P.S.E.B.M. 1991, Vol 197]

Diabetic neuropathy is attributed to a variety of structural and biochemical abnormalities within the nerve (1–5). The mechanisms responsible for all the diverse pathologic changes in the nerve are not completely understood. However, experimental evidence generated over the past two decades from animal and clinical studies has established a critical role for increased polyol pathway activity as an early key event initiating a cascade of biochemical, structural, and functional changes leading to neuropathy (3, 4).

Increased flux of glucose through the polyol pathway as a result of persistent hyperglycemia leads to an accumulation of sorbitol and fructose in tissues prone to develop diabetic complications (3, 4). In the sciatic nerve, increased polyol pathway activity appears to be responsible for myoinositol depletion and the derangement in phosphatidylinositol turnover that impairs Na^+,K^+-ATPase activity (4). Elevated polyol pathway activity (6–9) and an impairment in Na^+,K^+-ATPase activity (6–8) have been correlated with altered axolem-

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mal sodium permeability (9), early structural alterations at the nodes of Ranvier (8–10), and impaired nerve function (4, 6, 8–10). The fact that aldose reductase, the first enzyme in the polyol pathway, and Na^+,K^+-ATPase are both present in the nodal region of peripheral nerves (11, 12) provides evidence that the two biochemical pathways may be linked in the pathogenesis of diabetic neuropathy. Additional evidence for the role of aldose reductase in the pathogenesis of diabetic neuropathy is provided by pharmacologic studies in which prevention and early intervention treatment with structurally different aldose reductase inhibitors (ARI) has been shown to improve nerve function in diabetic rat (8, 13–16) and human diabetes (17, 18) and has beneficial effects on polyol pathway activity (8, 13–16, 18, 19), Na^+,K^+-ATPase activity (7, 8), and early structural lesions (8, 18, 19).

Although an earlier report showed that ARI treatment of streptozocin (STZ)-induced diabetic rats prevented the decline in Na^+,K^+-ATPase activity (7), a recent study did not confirm this observation (20). Both laboratories used a similar ATPase assay and to date the paradox has not been resolved. In the present study, we demonstrate that methodological differences affect the assessment of ARI efficacy. The original study, in which ARI treatment was shown to prevent the decline in Na^+,K^+-ATPase, was carried out in STZ-diabetic rats maintained on a diet supplemented with 68% sucrose (7). In the study in which ARI did not prevent the decline in Na^+,K^+-ATPase, the rats were maintained on normal chow (20). The nerve preparations used to quantitate ATPase activity also appear to be different in the two studies. Thus, in the present study we have used sorbinil and tolrestat, two structurally different ARI, and we have examined their effect on polyol pathway activity and on Na^+,K^+-ATPase activity using the different experimental conditions outlined in the two earlier, seemingly contradictory reports (7, 20).

Methods

Animals and Diet. Male Wistar rats weighing 230 ± 8 g (Charles River Breeding Laboratories, Kingston, NY) were used. To induce diabetes, streptozocin (Sigma Chemical Co., St. Louis, MO) dissolved in 0.2 ml of 0.01 mmol/liter citrate buffer (pH 5.5), was injected into the tail vein of rats fasted for 18 hr. Nondiabetic rats received 0.2 ml of buffer only. Two hours after STZ injection, rats were given access to either normal Purina Rodent Chow 5001-MZH or to a synthetic meal diet consisting of 68% sucrose, 18% casein, 0.011% free myoinositol, 10% vegetable oil, 4% inorganic salts, and all known rat vitamin requirements (Bio Serve, Frenchtown, NJ). The rats were maintained on their respective diets until completion of each study.

Two days after STZ injection and at the conclusion of each study, blood samples were collected from the tail vein. Rats were fasted 4 hr before sample collection to obviate postprandial peaks of glucose. Diabetic rats with nonfasting plasma glucose concentrations <16.5 mmol/liter were excluded from the study. Nondiabetic rats with plasma glucose concentrations >11 mmol/liter were also excluded.

Experimental Protocols. Three separate studies were performed—one study in which the rats were maintained on normal chow and two studies in which the rats were maintained on the 68% sucrose diet. In the normal chow study, one group of diabetic rats was given the ARI sorbinil at a dose of 20 mg/kg/day beginning on the day of STZ administration. After 4 weeks, blood samples were collected from the tail vein, the rats were sacrificed by CO_2 asphyxiation, and both sciatic nerves were removed and weighed. From each rat, one sciatic nerve was immediately processed for the measurement of ATPase activity; the contralateral nerve was quick frozen on dry ice and stored at −70°C until analyzed for carbohydrate and polyol content.

In the first sucrose chow study, tolrestat (25 or 50 mg/kg/day) was administered starting on the day of STZ administration. Rats were sacrificed at Week 6 and the nerves were processed as described above. The results of this study were used as a basis for the selection of the dose of tolrestat to be used in the intervention study outlined below.

The second sucrose chow study was designed to confirm the reported preventative effects of sorbinil (7) and to assess the effect of tolrestat in an intervention study. Starting on the day of STZ administration, one group of diabetic rats ($n = 10$) was treated with sorbinil (20 mg/kg/day). After 4 weeks, nonfasting plasma glucose concentrations were determined in all treatment groups, after which rats ($n = 6$) from each treatment group were sacrificed according to random plasma glucose values. The remaining rats in the nondiabetic rat group and the diabetic rat group were randomized into two additional groups according to plasma glucose values. Tolrestat was administered at a dose of 50 mg/kg/day to one group of nondiabetic rats and one group of diabetic rats. The study continued for an additional 4 weeks.

In all studies, the ARI were suspended in a volume of 1 ml of 2% Tween 80 in saline and administered every morning by gastric intubation. Control rats received 1 ml of vehicle.

Analytical Techniques. Plasma glucose concentrations were determined by a hexokinase method on an Abbot VP analyzer (Abbott Laboratories, Irving, TX). Sciatic nerve glucose, sorbitol, fructose, and myoinositol levels were determined by capillary gas chromatography, as described previously (21). Frozen tissues were homogenized with a Dounce tissue grinder in cold 5% trichloroacetic acid, and the deproteinized extracts were analyzed as their aldonitrile acetate derivatives. The

method has a detection limit of 0.04 nmol/mg tissue. Sciatic nerves from all three studies were analyzed at the termination of the last study. Results are expressed as mean nmol/mg wet weight ± SE.

Measurement of ATPase activity was carried out by the ATP-NADH coupled spectrophotometric assay, as described by Greene and Lattimer (7, 22). After removal, sciatic nerves were maintained on ice, cleaned of extraneous tissue and blood, and weighed. Each nerve was minced, suspended in cold 0.2 mol/liter sucrose, 0.02 mol/liter Tris buffer, pH 7.5 (homogenizing buffer), at a final concentration of 3.75 mg nerve/ml buffer. Nerve suspensions were homogenized at 4°C with either an Ultra-Turrax blade homogenizer equipped with a microprobe (Tekmar Co., Cincinnati, OH) or a motor-driven Dounce tissue grinder. In either procedure, the nerves were homogenized at 5000 rpm for three 15-sec periods. Homogenates were centrifuged at 100*g* for 2 min at 4°C to sediment large tissue debris; however, no pellet was observed with the Ultra-Turrax preparations. ATPase activity was determined in triplicate 5- to 15-μl samples of homogenate (2.5–5.0 μg of protein) added to 1.4 ml of reaction mixture. A partially purified preparation of dog kidney Na^+,K^+-ATPase (Sigma) suspended in homogenizing buffer was assayed daily as a control. The reaction mixture contained 100 mmol/liter NaCl, 10 mmol/liter KCl, 2.5 mmol/liter $MgCl_2$, 1 mmol/liter Tris-ATP, 1 mmol/liter tri(cyclohexylammonium) phosphoenol-pyruvate, 30 mmol/liter imidazole-HCl buffer (pH 7.3), 0.15 mmol/liter NADH, 50 μg/ml of lactate dehydrogenase (850 units/mg protein) and 30 μg/ml of pyruvate kinase (540 units/mg protein). After a stabilization period of 20 min, the oxidation of NADH was monitored for an additional 30 min. Na^+,K^+-ATPase was measured by comparing the reaction rates before and after the addition of ouabain to a final concentration of 0.1 mmol/liter. Preliminary experiments indicated that at a final protein concentration of 1.8–3.6 μg/ml in the assay, maximal inhibition of Na^+,K^+-ATPase activity was produced within the concentration range of 0.01–2.0 mmol/liter ouabain. To be consistent with the early studies by Greene and Lattimer (7, 22) and Das *et al.* (23), 0.1 mmol/liter ouabain was used in all measurements. The fraction of total activity inhibited by 0.1 mmol/liter ouabain (ouabain-sensitive) represented Na^+,K^+-ATPase activity. All reagents and enzymes were purchased from Sigma, and fresh solutions were prepared daily, except for the salt solutions, which were prepared weekly. The protein concentration was determined by the BCA Protein Assay (Pierce Chemical Co., Rockford, IL). ATPase activity was expressed as nmol/mg wet weight. When expressed as nmol/mg protein, results differed in magnitude but were otherwise consistent.

Statistical Analysis. Results are presented as mean ± SE. One- and two-way analyses of variance were performed. ATPase activities were not compared across studies because the samples were analyzed over a 4-month time period. All nerve carbohydrate and polyol levels were analyzed at the termination of the last study; in the 8-week longitudinal study, carbohydrate and polyol levels from Weeks 4 and 8 were compared.

Results

Body Weight and Plasma Glucose. Nondiabetic rats gained weight over the duration of the studies (Table I). Diabetic rats were hyperphagic and hyperglycemic for the duration of the studies and gained less weight than the nondiabetic rats. By Week 8, the diabetic rats lost an average of 10% of their original body weight. Beyond 6 weeks, there was high mortality (70%) in the diabetic group. Although comparisons were not made among studies, sucrose feeding did not appear to affect weight gain, food consumption, or plasma glucose levels. Administration of tolrestat or sorbinil did not affect body weight gain or plasma glucose levels.

Nerve Carbohydrate and Polyols. In the normal chow prevention study, 4 weeks of untreated diabetes resulted in an 8-fold increase in glucose, a 10-fold increase in sorbitol, a 12-fold increase in fructose, and an 18% lower myoinositol level (Table II). Sorbinil administration (20 mg/kg/day) completely prevented the rise in sorbitol and fructose and the depletion of myoinositol.

In the sucrose chow prevention studies, after 4 weeks of untreated diabetes, the sciatic nerves contained 7-fold higher levels of glucose, 18-fold higher levels of sorbitol, 11-fold higher levels of fructose, and 29% lower levels of myoinositol when compared with the nondiabetic controls. Administration of sorbinil (20 mg/kg/day) to diabetic rats completely prevented the rise in sorbitol and fructose and the depletion of myoinositol. When compared with the 4-week normal chow-fed diabetic rats, the sucrose-fed diabetic rats appeared to have higher sorbitol and lower myoinositol levels.

After 6 weeks, sciatic nerves from sucrose-fed diabetic rats contained 6-fold higher concentrations of glucose, 13-fold higher concentrations of sorbitol, 11-fold higher concentrations of fructose, and 47% lower concentrations of myoinositol when compared with the nondiabetic controls. Administration of tolrestat (25 or 50 mg/kg/day) to diabetic rats, prevented the rise in sorbitol and fructose and produced myoinositol levels that were 55% higher than the levels in the diabetic rats ($P < 0.01$) but below the concentration observed in the nondiabetic rats ($P < 0.01$).

In the nerves of 8-week sucrose-fed diabetic rats, there was a 10.3-fold increase in glucose, 18.6-fold increase in sorbitol, a 26.6-fold increase in fructose, and a 25% lower myoinositol level when compared

Table I. Terminal Body Weights and Plasma Glucose Values

	n	Body weight (g)	Plasma glucose (mmol/liter)
Normal chow prevention study			
4 weeks			
Nondiabetic	6	429 ± 5	5.7 ± 0.1
Diabetic	6	207 ± 10[a]	34.6 ± 2.2[a]
Diabetic + sorbinil (20 mg/kg)	6	241 ± 3[a]	29.8 ± 1.6[a]
Sucrose chow prevention studies			
4 weeks			
Nondiabetic	6	380 ± 8	6.0 ± 0.2
Diabetic	6	268 ± 7[a]	35.6 ± 2.1[a]
Diabetic + sorbinil (20 mg/kg)	6	266 ± 7[a]	37.5 ± 2.1[a]
6 weeks			
Nondiabetic	6	408 ± 6	5.5 ± 0.2
Diabetic	6	281 ± 7[a]	31.0 ± 1.4[a]
Diabetic + tolrestat (25 mg/kg)	6	249 ± 8[a]	27.4 ± 2.8[a]
Diabetic + tolrestat (50 mg/kg)	6	228 ± 6[a]	36.0 ± 4.3[a]
Sucrose chow intervention study			
8 weeks			
Nondiabetic	6	483 ± 10	6.3 ± 0.4
Nondiabetic + tolrestat[b] (50 mg/kg)	5	467 ± 7	5.8 ± 0.5
Diabetic	3	197 ± 19[a]	32.1 ± 0.7[a]
Diabetic + tolrestat[b] (50 mg/kg)	9	220 ± 11[a]	33.9 ± 2.3[a]

[a] $P < 0.01$ compared with nondiabetic.
[b] Tolrestat administered 4 weeks after the induction of diabetes.

Table II. Sciatic Nerve Sugar and Polyol Contents

	n	nmol/mg nerve			
		Glucose	Sorbitol	Fructose	Myoinositol
Normal chow prevention study					
4 weeks					
Nondiabetic	6	1.72 ± 0.20	0.10 ± 0.10	0.34 ± 0.01	3.52 ± 0.11
Diabetic	6	13.45 ± 1.12[a]	0.96 ± 0.04[a]	4.02 ± 0.44[a]	2.90 ± 0.08[a]
Diabetic + sorbinil (20 mg/kg)	6	13.29 ± 0.89[a]	0.16 ± 0.08[b]	0.53 ± 0.02[b]	3.70 ± 0.23[b]
Sucrose chow prevention studies					
4 weeks					
Nondiabetic	6	1.71 ± 0.09	0.15 ± 0.02	0.36 ± 0.06	2.14 ± 0.15
Diabetic	6	11.48 ± 0.59[a]	2.68 ± 0.11[a]	4.00 ± 0.28[a]	1.51 ± 0.08[a]
Diabetic + sorbinil (20 mg/kg)	6	13.00 ± 0.56[a]	0.76 ± 0.07[a,b]	1.92 ± 0.18[a,b]	2.00 ± 0.14[b]
6 weeks					
Nondiabetic	6	2.06 ± 0.07	0.20 ± 0.02	0.62 ± 0.13	2.51 ± 0.30
Diabetic	6	12.51 ± 0.49[a]	2.66 ± 0.33[a]	6.75 ± 0.36[a]	1.32 ± 0.08[a]
Diabetic + tolrestat (25 mg/kg)	6	17.23 ± 0.90[a]	0.24 ± 0.03[b]	1.09 ± 0.38[b]	2.08 ± 0.12[b,c]
Diabetic + tolrestat (50 mg/kg)	6	15.78 ± 0.83[a]	0.21 ± 0.02[b]	0.58 ± 0.06[b]	2.02 ± 0.03[a,b]
Sucrose chow intervention study					
8 weeks					
Nondiabetic	6	1.57 ± 0.16	0.15 ± 0.02	0.27 ± 0.04	2.75 ± 0.12
Nondiabetic + tolrestat[d] (50 mg/kg)	5	1.51 ± 0.16	ND[e]	0.12 ± 0.10	2.57 ± 0.15
Diabetic	3	16.23 ± 2.37[a]	2.79 ± 0.50[a]	7.17 ± 0.55[a]	2.06 ± 0.19[a]
Diabetic + tolrestat[d] (50 mg/kg)	9	17.90 ± 1.26[a]	0.02 ± 0.02[b]	0.26 ± 0.06[b]	3.08 ± 0.22[b]

[a] $P < 0.01$ compared with nondiabetic.
[b] $P < 0.01$ compared with diabetic.
[c] $P < 0.05$ compared with nondiabetic.
[d] Tolrestat administered 4 weeks after the induction of diabetes.
[e] ND, not detected.

with the nondiabetic control. When compared with the 4-week sucrose-fed diabetics, the sorbitol concentration was comparable whereas the glucose, fructose, and myoinositol levels were higher in the 8-week diabetic rats ($P < 0.01$). An increase or normalization of nerve myoinositol levels with the duration of diabetes has been reported previously (16). Intervention treatment with tolrestat 4 weeks after the induction of diabetes and continuous treatment for 4 subsequent weeks completely normalized the sorbitol, fructose, and myoinositol levels.

Nerve ATPase Activity. In the normal chow study, a 4-week duration of diabetes resulted in a 40% reduction in both the composite and ouabain-resistant ATPase fractions and a 54% reduction in Na^+,K^+-ATPase activity (Table III). Sorbinil treatment did not prevent the decline in enzyme activity.

In the 4-week sucrose chow prevention study, diabetes induced a 22% lower composite ATPase activity, a 20% lower ouabain-resistant activity, and a 26% lower Na^+,K^+-ATPase activity when compared with the controls. Sorbinil (20 mg/kg/day) completely prevented the decline in all three ATPase enzyme fractions.

After 6 weeks, the sciatic nerves from sucrose-fed diabetic rats had 25% lower composite and ouabain-resistant ATPase activities and 27% lower Na^+,K^+-ATPase activity, when compared with the control. Administration of tolrestat at a dose of 25 mg/kg/day did not prevent the diabetes-induced decline in ATPase activity. However, at a dose of 50 mg/kg/day, tolrestat effectively prevented the decline in composite ATPase activity ($P < 0.05$ compared with diabetic) and Na^+,K^+-ATPase activity ($P < 0.01$ compared with diabetic; not significant compared with nondiabetic).

In 8-week sucrose-fed diabetic rats, the composite, ouabain-resistant, and Na^+,K^+-ATPase activities were 33%, 34%, and 28% lower, respectively, when compared with the age-matched controls. Four weeks of tolrestat intervention treatment did not affect the ouabain-resistant ATPase fraction, but did result in a 11% higher composite ATPase activity and a 20% higher Na^+,K^+-ATPase when compared with the diabetic rats. Administration of tolrestat to nondiabetic rats did not affect ATPase activity.

Discussion

The study presented here demonstrates that ARI can effectively prevent the decline in sciatic nerve Na^+,K^+-ATPase activity in STZ-diabetic rats when administered preventatively or as intervention therapy

Table III. ATPase Activities in Sciatic Nerves

	n	ATPase activity (μmol ADP produced/g nerve/hr)		
		Composite	Ouabain-sensitive	Ouabain-resistant
Normal chow prevention study[a]				
4 weeks				
Nondiabetic	6	526.9 ± 21.9	88.2 ± 15.7	438.7 ± 16.5
Diabetic	6	305.7 ± 10.1[b]	40.7 ± 5.7[b]	265.0 ± 10.1[b]
Diabetic + sorbinil (20 mg/kg)	6	319.2 ± 30.8[b]	34.4 ± 7.4[b]	284.8 ± 8.2[b]
Sucrose chow prevention studies[c]				
4 weeks				
Nondiabetic	6	1371.2 ± 47.8	322.3 ± 18.4	1048.9 ± 35.1
Diabetic	6	1076.0 ± 24.5[b]	238.6 ± 15.7[b]	837.5 ± 16.2[b]
Diabetic + sorbinil (20 mg/kg)	6	1326.2 ± 50.7[d]	321.5 ± 21.7[d]	1006.7 ± 38.8[d]
6 weeks				
Nondiabetic	6	1128.4 ± 29.2	275.0 ± 11.9	853.4 ± 23.8
Diabetic	6	846.3 ± 24.4[b]	199.7 ± 8.4[b]	646.6 ± 21.3[b]
Diabetic + tolrestat (25 mg/kg)	6	928.5 ± 28.9[b]	215.9 ± 10.5[b]	712.6 ± 20.0[b]
Diabetic + tolrestat (50 mg/kg)	6	957.4 ± 23.0[b,e]	242.6 ± 12.1[d]	714.8 ± 16.4[b]
Sucrose chow intervention study				
8 weeks				
Nondiabetic	6	1196.1 ± 23.9	250.1 ± 13.1	946.0 ± 22.3
Nondiabetic + tolrestat[f] (50 mg/kg)	5	1192.3 ± 23.9	266.9 ± 9.4	925.5 ± 23.6
Diabetic	3	807.0 ± 18.3[b]	179.4 ± 6.5[b]	627.6 ± 17.7[b]
Diabetic + tolrestat[f] (50 mg/kg)	9	895.9 ± 11.6[b,e]	214.5 ± 6.9[e]	681.5 ± 9.3[b]

[a] In the normal chow study, nerves were homogenized with a Dounce homogenizer.
[b] $P < 0.01$ compared with nondiabetic.
[c] In sucrose chow studies, nerves were homogenized with an Ultra-Turrax.
[d] $P < 0.01$ compared with diabetic.
[e] $P < 0.05$ compared with diabetic.
[f] Tolrestat administered 4 weeks after the induction of diabetes.

under certain experimental conditions. ARI were found to effectively prevent the diabetes-induced decline in Na^+,K^+-ATPase activity when the STZ-diabetic rats were maintained on a diet supplemented with 68% sucrose and ATPase activity was measured in nerve homogenates prepared with an Ultra-Turrax disperser. However, in STZ-diabetic rats fed normal rodent chow, and in which nerve homogenates were prepared with a Dounce tissue grinder, ARI treatment did not prevent the decline in Na^+,K^+-ATPase activity. The findings in the present study confirm the findings of two earlier and seemingly contradictory reports that used the distinct experimental conditions outlined above (7, 20). We propose that the paradox in ARI efficacy was due to a difference in the sciatic nerve preparation used to measure ATPase activity and/or a difference in the rodent diet.

In the normal chow study, which was the first study conducted in this investigation, the sciatic nerves were homogenized with a Dounce tissue grinder, which is similar to the method used routinely by Lambourne *et al.* (20, 24). Upon finding no beneficial effect of sorbinil on Na^+,K^+-ATPase activity, subsequent studies were initiated in which the methodology of Greene and Lattimer (7, 22) was followed closely. The rats were maintained on a diet consisting of 68% sucrose and the sciatic nerves were homogenized with a blade homogenizer, the Ultra-Turrax disperser. Although we followed the procedure described by Greene and Lattimer (7, 22), the ATPase activities observed in the Ultra-Turrax prepared homogenates in the present study were higher than those reported in the study by Greene and Lattimer (7), in which a Polytron was used. In their report, ATPase activity was measured in the homogenate after low-speed centrifugation to remove large pieces of tissue debris. In the present study, no pellet was observed after low-speed centrifugation, suggesting a more thorough homogenization of the nerve.

Higher ATPase activities in all Ultra-Turrax preparations were correlated with higher total protein concentrations, indicating an increase in the concentration of enzyme rather than an increase in the catalytic efficiency of the enzyme. The difference in protein concentrations between the two homogenization techniques was not unexpected, inasmuch as blade homogenizers (Ultra-Turrax and Polytron) release more total protein from tissue and are the method of choice for fibrous tissue (25). To ensure that the higher enzyme values were not due to the sucrose diet, nerves from nondiabetic sucrose-fed rats were homogenized with a Dounce tissue grinder; the Na^+,K^+-ATPase activity was found to be low (89.7 ± 10.1 μmol ADP/g nerve/hr ($n = 6$)) and nerve preparations from normal chow-fed rats prepared with the Ultra-Turrax had high enzyme activity (301.4 ± 16.5 μmol ADP/g nerve/hr ($n = 6$)). Therefore, it was the use of the Ultra-Turrax and not the sucrose diet that was responsible for the higher ATPase activity.

A difference in the amount of ATPase released from the nerve by the two different homogenization techniques may account for the disparity in the magnitude of the decline in Na^+,K^+-ATPase activity in the diabetic rats fed sucrose (26% decline, Ultra-Turrax) versus the diabetic rats fed standard chow (54% decline, Dounce). Additionally, it is conceivable that the beneficial effect of ARI on Na^+,K^+-ATPase activity is the consequence of the thorough homogenization and release of an ARI-sensitive ATPase fraction by the Ultra-Turrax. Llewelyn *et al.* (26) have reported that an ARI-sensitive Na^+,K^+-ATPase resides in the fibrous perineurium, but not the endoneurium, of the sciatic nerve. However, they examined sciatic nerves from galactose-fed rats, which may not be relevant to diabetes since increased polyol pathway activity in this model is associated with a contradictory increase in neural Na^+,K^+-ATPase activity (24, 26).

Although differences in tissue processing may have affected the outcome of this and previous studies (7, 20), it is also possible that the sucrose diet induced a pathogenesis in the diabetic nerve, whereby ARI were effective in preventing the Na^+,K^+-ATPase decline. Administration of high dietary sucrose or fructose to diabetic rats is known to exacerbate tissue glucose levels, which in turn enhances polyol pathway activity (27–29), increases microangiopathy (28, 30–32), and accelerates the development of diabetic complications (29–32). Additionally, high dietary sucrose induces systemic hormonal (33, 34) and metabolic (33, 35) alterations, which have been shown to either directly or indirectly affect Na^+,K^+-ATPase activity in a variety of tissues (36, 37).

High dietary sucrose did appear to exacerbate polyol pathway activity in the diabetic rats in the present report, although statistical comparisons were not made among the individual studies. Alone, high dietary sucrose has been shown to produce vascular changes (30, 32) and endoneurial edema (38) in nondiabetic rats. In the present study, the sucrose-fed nondiabetic rats appeared to have a lower neural myoinositol concentration than the chow-fed nondiabetic rats. The levels of myoinositol in the 4-, 6-, and 8-week sucrose-fed nondiabetic rats (2.14, 2,51, and 2.75 nmol/mg, respectively) were only slightly lower than those reported previously for nondiabetic rats fed an identical diet for 4 weeks (2.79 nmol/mg) (7). A deficiency or excess of dietary myoinositol is known to affect the neural myoinositol concentration (22, 39); however in the present study, both diets were within the normal range of dietary myoinositol (22, 39), i.e., 0.011% for the sucrose diet and 0.035% for the Purina Rodent Chow. Therefore, why there might be a lower neural myoinositol

concentration in the sucrose-fed nondiabetic rats cannot be explained at this time.

High dietary sucrose accelerates the development of microangiopathy in a variety of tissues (28, 30–32). In the peripheral nerve, there is evidence that occlusion of the microvasculature leads to hypoxia, which in turn impairs a variety of ATP-dependent processes, including Na^+,K^+-ATPase (40). Thus, it is possible that in the sucrose-fed diabetic rats, hypoxia, as a consequence of advanced microvascular occlusion (29), may be the primary cause of the decline in nerve Na^+,K^+-ATPase in this model. Moreover, aldose reductase appears to play a central role in this pathologic process, since aldose reductase has been localized in a variety of cells of the microvasculature (11) and ARI have been shown to prevent basement membrane thickening (41, 42) and increased capillary permeability (43). Thus taking into consideration the microvascular alterations associated with high dietary sucrose and the proven effectiveness of ARI in preventing microangiopathy, it is likely that the administration of high dietary sucrose to diabetic rats may have induced an ARI-preventable occlusion of the nerve microvasculature that impaired Na^+,K^+-ATPase and that would account for the difference in ARI efficacy between the two diet models.

In addition to histopathologic changes (30–32), administration of high dietary sucrose to rodents induces hormonal and metabolic changes that are common to diabetic humans, including hyperinsulinemia (33, 34), alterations in thyroid hormone metabolism (34), abnormal glucose and lipid metabolism (33, 35), and elevated Na^+,K^+-ATPase activity in tissues such as liver and muscle (36). The rise in Na^+,K^+-ATPase activity was presumably due to hormonal mediators or activation of the sympathetic nervous system (36, 37). Thus, with all the potential confounding metabolic and hormonal effects of high dietary sucrose, there may be unanticipated direct or indirect effects on the sciatic nerve which could affect the pathogenesis and treatment of diabetic neuropathy.

Reports by Lambourne *et al.* (20, 24) indicate that the relationship between aldose reductase activity, myoinositol depletion, and the deficit in neural Na^+,K^+-ATPase activity is not as clear-cut as proposed originally (4). The current findings support those early studies. First, in the studies in which the STZ-diabetic rats were maintained on normal rodent chow, i.e., the present report and the report by Lambourne *et al.* (20), there was a clear dissociation between the polyol pathway activity and Na^+,K^+-ATPase activity. ARI completely normalized the sorbitol and myoinositol levels, but were without effect on Na^+,K^+-ATPase activity. Second, in the present sucrose-fed diabetic rat study, the dose of tolrestat which prevented the decline in Na^+,K^+-ATPase activity (50 mg/kg/day) was 2-fold higher than the dose which prevented the changes in sorbitol, fructose, and myoinositol. This difference in potency suggests that (i) the effect of an ARI on the polyol pathway is not tightly associated with its effect on Na^+,K^+-ATPase and/or (ii) total nerve polyol measurements may not accurately reflect discrete changes in highly compartmentalized biochemical events such as phosphatidylinositol turnover, which may be correlated more closely with Na^+,K^+-ATPase activity (3, 44).

The current results confirm the early finding by Greene and Lattimer (7) that shows that an ARI prevents the decline in sciatic nerve Na^+,K^+-ATPase activity in the STZ-diabetic rat, and also confirm the more recent finding by Lambourne *et al.* (20) that shows no beneficial effect on Na^+,K^+-ATPase. The findings in the present report provide strong evidence that the reported contradiction in ARI efficacy is due to (i) differences in the nerve preparations used to measure ATPase activity and/or (ii) a dietary-induced difference in the etiology of the Na^+,K^+-ATPase impairment. The data also indicate that the effect of an ARI in ameliorating the Na^+,K^+-ATPase deficit is not tightly associated with its effect on polyol pathway activity and perhaps reflects the action of the compound on a variety of regions in the nerve. Furthermore, the impairment in Na^+,K^+-ATPase activity in the complex sucrose-fed diabetic rat model may not be due simply to polyol pathway activity and/or ischemia, but may also involve metabolic and hormonal changes that accompany sucrose feeding.

Although the findings in the present study resolve the apparent contradiction of earlier reports (7, 20), there remain many questions concerning the precise mechanisms that contribute to the diabetes-induced decline in neural Na^+,K^+-ATPase. Clearly, more work needs to be done.

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