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Prevention of Neural Myoinositol Depletion in Diabetic Rats by Aldose Reductase Inhibition with Tolrestat[1] (42372)

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Abstract. The effect of the aldose reductase inhibitor tolrestat on the sugar and polyol contents in the sciatic nerve was investigated in male Wistar and Sprague–Dawley rats rendered diabetic with streptozocin. At a daily oral dose of 5 mg/kg, given for 10 days before and for 14 days after streptozocin injection, tolrestat completely prevented the accumulation of sorbitol and the depletion of myoinositol.

Decreased myoinositol levels in sciatic nerves were first observed by Stewart *et al.* in rats with experimentally induced diabetes or galactosemia (1). Tentatively, the authors attributed the decrease to the osmotic effect of neural galactitol accumulation, which causes cell membrane damage, thus affecting the uptake and retention of myoinositol (2). The postulate was based on the recognition that the osmotic stress produced by activation of the polyol pathway represents the pivotal event in the pathogenesis of sugar cataract (3), and was consonant with the finding of the polyol pathway in the lens (4–6) and peripheral nerves (7–9), and with the inverse relationship between lens, glucose, and myoinositol levels occurring in human cataract (10).

The current interest in neural myoinositol (11) was prompted by the demonstration that decreases in myoinositol levels and conduction velocity in the peripheral nerves of diabetic rats could be prevented by adding 1% myoinositol to the diet or by insulin treatment (12). The finding inspired the postulate that discrete, small neural pools of phosphatidylinositol with rapid turnover (13) serve as mechanisms of metabolic regulation (14). Since relatively high neural myoinositol levels (15) were regarded as necessary to ascertain prompt phosphatidylinositol resynthesis, it was concluded that hyperglycemia affects peripheral nerve metabolism by impairing the maintenance of adequate neural myoinositol levels (14). The finding that the diabetes-induced decrease in neural Na,K-dependent ATPase activity (16) was prevented by feeding rats 1% myoinositol with the diet (17) has led to the hypothesis that in the nerve, the Na-dependent myoinositol uptake and the myoinositol-related Na,K-ATPase are linked by a self-enforcing cycle (18, 19). Data on the effect of orally administered myoinositol on peripheral nerve function in human diabetic patients are, however, inconclusive (20–26). That the described metabolic abnormalities and impaired function in the diabetic nerve are, in fact, associated with the increased flux of glucose through the polyol pathway was demonstrated in rats by inhibiting aldose reductase (AR) (27, 28).

We have recently described tolrestat, a novel, orally active, nonhydantoin AR inhibitor (29), and reported on its pharmacodynamics (30–32) and pharmacokinetics (33, 34) in laboratory animals and man. We relate herewith the effect of tolrestat on the glucose and myoinositol metabolism in the sciatic nerve of rats with streptozocin-induced diabetes. Since rats of the Wistar strain were used in all reported studies on neural myoinositol metabolism (12, 28, 35–42), we considered it of interest to use rats of both the Wistar and Sprague–Dawley strains.

Materials and Methods. *Animals.* Male, cesarean section-delivered Wistar and Sprague–Dawley rats weighing 140–180 g were used (Charles River Breeding Laboratory, Wilmington, Mass.). The animals had unlimited access to water and a diet containing 0.01% myoinositol, 68% sucrose, 18% casein, 10% vegetable oil, 4% inorganic salts, and rat vitamin supplements (Bioserve, Frenchtown, N.J.). Such a diet was originally used by Greene *et al.* (12) since sciatic nerve myoinositol concentrations depend on the myoinositol content in the diet.

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After an overnight fast, diabetes was induced by a single injection of streptozocin (STZ) at a dose of 55 mg/kg in 0.2 ml of 0.03 *M* citrate buffer, pH 4.5, into the tail vein. Nondiabetic rats, used as control, received equal volumes of buffer alone. For protection against hypoglycemia occurring after the STZ injection, as needed, rats were given access to drinking water containing 10% glucose. Animals with nonfasting plasma glucose concentrations below 300 mg/dl (16.5 mmole/liter) and above 800 mg/dl (43.9 mmole/liter) at 48 hr after STZ injection were discarded from the study.

Tolrestat was dissolved in saline containing 2% Tween 80 in amounts to provide doses of 5, 10, and 20 mg/kg, administered once daily by gastric intubation in a volume of about 1 ml. Since the effectiveness of tolrestat can be increased by pretreatment (30, 31), the animals received tolrestat for 10 days before, and for 14 days after, the STZ injection. At that time, the rats were anesthetized with halothane and killed by exsanguination; the mid-thigh segments of the sciatic nerve were surgically removed, weighed, and frozen on dry ice. The blood was transferred to a heparinized tube and centrifuged; the plasma was removed and frozen. Frozen nerve samples were homogenized in 5% trichloroacetic acid and centrifuged; the separated supernatant was neutralized with 2 *N* aqueous KOH, centrifuged again, frozen on dry ice, and lyophilized. The same extraction procedure was used with plasma to determine myoinositol concentrations when the animals were killed.

Analytical methods. Plasma glucose in tail-vein blood samples was determined on the Abbott VP chemistry analyzer. Sciatic nerve glucose, sorbitol, fructose, myoinositol, and plasma myoinositol were determined by capillary gas chromatography of the lyophilized aqueous extracts by a modification of the method of Guerrant and Moss (43). A Hewlett–Packard 5890 gas chromatograph was used, with a 30 m × 0.25-mm SP-2100 (Supelco) capillary column (programmed for 4 min at 175°C, and then for an increase of 4°C/min to 270°C) using a flame ionization detector at 250°C. The reported values represent means ± standard error of the mean.

Statistical analysis. One- and two-way analyses of variance were performed. A probability level of 0.05 or less was considered significant.

Results. *Body weight, plasma glucose, and myoinositol (Table I).* At the end of the 4-week study period, all diabetic rats of both strains showed hyperglycemia and decreased body weight gain. Treatment with tolrestat had no effect on either variable in normal rats. Neither STZ-diabetes (12) nor tolrestat affected plasma myoinositol concentrations. Two of 14 samples in the 10 mg/kg group were, however, contaminated, resulting in a higher average myoinositol concentration. No particular differences in plasma glucose and myoinositol concentrations were apparent between the two strains of rats. The high group-average plasma glucose levels in diabetic Wistar rats treated with tolrestat were due to atypically high values (40.6–43.6 mmole/liter) found in two of eight animals in the 5 mg/kg group, and in one of seven animals of the 10 mg/kg group; such variation is not unusual in diabetic rats. The fact that similar plasma glucose concentrations were found in the diabetic control and highest tolrestat dose groups excludes the possibility that the effects were drug related.

Neural sugar and polyol levels (Table II). As expected, all untreated diabetic animals showed in their sciatic nerves a marked increase in glucose and massive accumulation of sorbitol and fructose, associated with the typical 25–30% reduction in myoinositol levels. Treatment of the diabetic animals with tolrestat at a dose of 5 mg/kg prevented both the accumulation of neural sorbitol and fructose, and the depletion of neural myoinositol content. With the experimental conditions used, tolrestat was thus more efficacious than reported earlier (ID_{50} of 4.8 mg/kg/day for the sciatic nerve) (30).

Treatment with the two lower doses of tolrestat appeared to increase the neural glucose levels in diabetic rats of both strains. The significance of the finding is questionable since it was not evident after the highest dose of tolrestat. Increased glucose content in the sciatic nerve of STZ-induced diabetic Wistar rats was occasionally found upon treatment with the aldose reductase inhibitors sorbinil (20 mg/kg/day) (44) and statil (25 mg/kg/day) (45).

Tolrestat administration to nondiabetic animals had no effect on the sciatic nerve sorbitol and myoinositol levels; with respect to fructose

TABLE I. EFFECTS OF TOLRESTAT AND STREPTOZOCIN-DIABETES ON BODY WEIGHT CHANGE AND TERMINAL PLASMA GLUCOSE AND MYOINOSITOL CONCENTRATIONS IN WISTAR AND SPRAGUE–DAWLEY RATS

Strain	Group	N	Body weight (g)		Plasma	
			Week 0	Week 4	Glucose (mmole/liter)	Myoinositol (μmole/liter)
Wistar	Nondiabetic	17	173 ± 2.3	298 ± 4.9	8.72 ± 0.14	22.6 ± 0.9
	Nondiabetic + tolrestat (10 mg/kg)	16	173 ± 2.5	294 ± 5.1	8.45 ± 0.17	19.0 ± 0.6
	Diabetic	17	177 ± 2.5	213 ± 6.1*	28.58 ± 0.89	24.1 ± 1.3
	Diabetic + tolrestat					
	5 mg/kg	8	177 ± 4.3	205 ± 6.7*	33.64 ± 2.17**	21.9 ± 2.7 (7)
	10 mg/kg	7	179 ± 1.7	212 ± 12.5*	32.17 ± 1.87**	22.5 ± 1.6
	20 mg/kg	9	172 ± 2.0	190 ± 6.1*	27.83 ± 1.15	23.1 ± 2.8 (8)
Sprague–Dawley	Nondiabetic	16	158 ± 3.8	305 ± 7.0	9.22 ± 0.16	23.0 ± 2.2 (15)
	Nondiabetic + tolrestat (10 mg/kg)	17	159 ± 3.1	303 ± 5.1	9.05 ± 0.11	19.2 ± 1.8 (13)
	Diabetic	19	155 ± 3.3	197 ± 6.4*	29.25 ± 0.66	21.4 ± 1.8 (18)
	Diabetic + tolrestat					
	5 mg/kg	15	157 ± 2.7	188 ± 6.9*	32.05 ± 1.20	25.4 ± 1.4
	10 mg/kg	15	160 ± 3.1	189 ± 4.5*	30.63 ± 1.26	35.5 ± 6.7**
	20 mg/kg	11	160 ± 3.9	181 ± 7.0*	29.64 ± 0.99	23.8 ± 2.5

Note. Diabetes was induced by streptozocin (55 mg/kg iv). Drug-treated rats were administered tolrestat once daily by gastric intubation at the doses indicated, for 10 days before, and for 14 days after, injection of streptozocin. Number of samples analyzed is in parentheses. Values are given as means ± SEM. Massive differences from the appropriate reference group were not statistically tested.

* $p < 0.05$ as compared to nondiabetic control.

** $p < 0.05$ as compared to diabetic control.

TABLE II. EFFECTS OF TOLRESTAT AND STREPTOZOCIN-DIABETES ON SCIATIC NERVE CONTENTS OF GLUCOSE, SORBITOL, FRUCTOSE, AND MYOINOSITOL IN WISTAR AND SPRAGUE–DAWLEY RATS

Strain	Group	N	Sciatic nerve contents (nmole/mg ± SE)			
			Glucose	Sorbitol	Fructose	Myoinositol
Wistar	Nondiabetic	17	2.49 ± 0.18	0.12 ± 0.04	<0.64	3.00 ± 0.18
	Nondiabetic + tolrestat (10 mg/kg)	16	3.95 ± 0.87	<0.08	<0.64	3.72 ± 0.24
	Diabetic	17	13.52 ± 1.11	1.77 ± 0.20	3.49 ± 0.48	2.26 ± 0.10*
	Diabetic + tolrestat					
	5 mg/kg	8	26.04 ± 1.30**	0.13 ± 0.04	<0.64	3.59 ± 0.19
	10 mg/kg	7	19.51 ± 1.25**	<0.08	<0.64	3.25 ± 0.15
	20 mg/kg	9	16.36 ± 0.85	<0.08	<0.64	3.30 ± 0.19
Sprague–Dawley	Nondiabetic	16	2.04 ± 0.19	<0.16	<0.61	3.35 ± 0.22
	Nondiabetic + tolrestat (10 mg/kg)	17	2.00 ± 0.29	<0.16	***	3.47 ± 0.47
	Diabetic	19	7.81 ± 0.43	1.06 ± 0.12	4.35 ± 0.65	2.42 ± 0.09*
	Diabetic + tolrestat					
	5 mg/kg	15	10.54 ± 0.52**	<0.16	0.88 ± 0.16**	3.07 ± 0.13
	10 mg/kg	15	10.89 ± 0.92**	<0.16	0.99 ± 0.23**	3.56 ± 0.20
	20 mg/kg	11	8.91 ± 0.87	<0.16	<0.61	3.36 ± 0.16

Note. Animals treated as described in Table 1.

* $P < 0.05$ as compared to nondiabetic control.

** $P < 0.05$ as compared to diabetic control.

*** Results not available.

levels, an unexpected contamination detected in 9 of 17 nondiabetic Sprague–Dawley rats resulted in a meaningless high-average value, and the value is not included.

Discussion. The results obtained in the present study demonstrate that at an oral dose of 5 mg/kg/day,[2] tolrestat prevented the accumulation of sorbitol and fructose and the depletion of myoinositol in the sciatic nerve of 10-day diabetic rats of both Wistar and Sprague–Dawley strains. In accordance with Greene *et al.* (12), we have fed rats on a diet containing 0.01% myoinositol and 68% sucrose; since in STZ-induced diabetic rats feeding on a sucrose-rich diet accelerates the appearance of retinopathy (47), it is possible that it also affects the development of neural changes.

Our results confirm that the hyperglycemia-induced accumulation of sorbitol and fructose and the depletion of myoinositol in the sciatic nerve of diabetic rats are prevented by suppressing aldose reductase activity with an effective inhibitor. The fact that similar results were obtained with four structurally distinct AR inhibitors (36, 38, 41, 42) provides firm evidence that the observed effect in the sciatic nerve is indeed caused by inhibition of aldose reductase and not by some other pharmacological activity.

In addition to providing a rational basis for pharmacological intervention (48), the results obtained with AR inhibitors have exposed the critical role of aldose reductase in the development hyperglycemia-induced biochemical, functional, and structural deficits in the diabetic nerve. While unclear, at present, which process(es) link the enhanced aldose reductase activity with myoinositol depletion in the diabetic nerve, it is very likely that the critical event is accumulation of neural sorbitol rather than inhibition of neural myoinositol uptake by glucose (49, 50). The possibility thus remains that localized aldose reductase produces foci of high sorbitol accumulation which gradually lead to functional disturbances, e.g., of the membrane-bound Na,K-dependent ATPase (16).

[2] Complete prevention of myoinositol depletion was also effected in the sciatic nerve of rats with severe chronic galactosemia and treated with tolrestat at a daily dose of 7 mg/kg (46).

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