An Aldose Reductase Inhibitor But Not *myo*-Inositol Blocks Enhanced Polyphosphoinositide Turnover in Peripheral Nerve From Diabetic Rats

Liliana Berti-Mattera, Nancy Day, Richard G. Peterson, and Joseph Eichberg

Experimental diabetic neuropathy, whether chemically induced or present in several spontaneously diabetic animal models, is characterized by sorbitol accumulation and *myo*-inositol depletion and usually also by enhanced turnover of the monoesterified moieties of polyphosphoinositides, particularly phosphatidylinositol-4,5-bisphosphate (PIP_2). This study examined the relationship of these alterations by assessing the effects of *myo*-inositol and the aldose reductase inhibitor, sorbinil, supplied as dietary supplements, on sorbitol and *myo*-inositol concentrations and incorporation of ^{32}P into polyphosphoinositides in sciatic nerve from rats killed 8 weeks after induction of diabetes with streptozotocin. Nerves from diabetic rats killed after 8 weeks of disease exhibited 52% to 76% greater PIP_2 labeling, markedly elevated sorbitol levels, and 30% less *myo*-inositol when compared with age-matched normal rats. Incorporation of isotope into PIP_2 in nerves from animals fed a *myo*-inositol supplement, added to either a high-sucrose diet or standard rat chow beginning immediately after induction of diabetes, remained substantially elevated, whereas *myo*-inositol levels were corrected to normal. Essentially the same results were obtained when rats were fed the *myo*-inositol–containing diet beginning 4 weeks after streptozotocin injection. In contrast, PIP_2 labeling in nerves from diabetic rats that received the sorbinil-supplemented diet for either 4 or 8 weeks was not different from that in controls. *myo*-Inositol levels in these animals were also restored to normal, whereas sorbitol levels remained elevated, albeit reduced by approximately 30%. These results indicate that *myo*-inositol administration is unable to completely counteract the impact of diabetes on the turnover of monoesterified phosphate groups in PIP_2. In contrast, sorbinil can correct this abnormality, but this beneficial effect is not dependent on the presence of normal sorbitol concentrations.

NUMBER OF DEFECTS in peripheral nerve function are associated with experimental diabetes, of which the most readily detected and widely measured is abnormally low nerve conduction velocity. Well-characterized biochemical aberrations include increased sorbitol and fructose levels, decreased *myo*-inositol content, and diminished Na^+-pump activity, measured either as Na^+,K^+-adenosine triphosphatase (ATPase) activity in nerve preparations or as uptake of ^{86}Rb into the intact tissue.[1-3] It has been demonstrated that administration of aldose reductase inhibitors to diabetic animals can simultaneously ameliorate the abnormalities in carbohydrate concentrations and restore Na^+,K^+-ATPase activity and conduction velocity to normal.[1,4,5] A similar correction pattern is elicited by dietary supplementation with *myo*-inositol.[3,4]

One hypothesis that links the reduced *myo*-inositol levels to altered Na^+,K^+-ATPase activity is that decreased activity of the phosphoinositide cycle limited by the supply of this cyclitol will reduce phosphoinositidase C–catalyzed production of the second messengers, 1,2-diacylglycerol and inositol 1,4,5-trisphosphate, and thereby diminish protein kinase C activity.[1,6] The decreased action of this enzyme is proposed to downregulate the Na^+ pump and consequently Na^+-dependent *myo*-inositol uptake. The validity of this scheme is in doubt because, although there is evidence for decreased phosphatidylinositol-4,5-bisphosphate (PIP_2) breakdown and reduced 1,2-diacylglycerol content in diabetic nerve,[7-9] controversy exists as to whether protein kinase C activity is elevated or reduced and how this enzyme may influence the Na^+ pump.[10-14] It is likely that more consideration will be needed both of the metabolic compartmentation within the nerve and of the role of individual protein kinase C isoforms before this hypothesis can be further evaluated.

In addition to the degradation of PIP_2 by phosphoinositidase C, the monoesterified phosphate moieties of this phospholipid are also well known to undergo rapid turnover by the concerted action of phosphatases and kinases.[15] Although the physiological significance of this substrate cycle remains to be elucidated, it has been hypothesized that in nervous tissue one function for the turnover of phosphate groups is related to the maintenance of the myelin sheath, in which PIP_2 is primarily located.[16,17] In several diabetic rodent models, including the streptozotocin-induced model and two genetically diabetic rat strains, Wistar and Zucker diabetic fatty rats, turnover of PIP_2 as judged by incorporation of ^{32}P into phospholipids via this substrate cycle in nerve is enhanced by a mechanism that does not involve increased specific activity of tissue ATP.[18-21] This metabolic alteration is manifested soon after induction of diabetes and persists for many months thereafter.[22] Increased turnover of PIP_2 was not detected in the *db/db* mouse, a diabetic model in which changes in nerve sorbitol and *myo*-inositol have not been detected.[23,24]

Thus, it appears that heightened metabolism of monoesterified phosphate moieties of PIP_2 may be a correlate of the onset of diabetic neuropathy in animal models that also display altered sorbitol and *myo*-inositol levels in nerve.

In this study, we investigated the possible relationship

From the Department of Biochemical and Biophysical Sciences, University of Houston, Houston, TX; and the Department of Anatomy, Indiana University Medical School, Indianapolis, IN.

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Present address: L.B.-M., Division of Hypertension and Endocrinology, Department of Medicine, Case Western Reserve University Medical School, Cleveland, OH 44106; N.D., Department of Medicine, Baylor College of Medicine, Houston, TX 77030.

Address reprint requests to Joseph Eichberg, PhD, Department of Biochemical and Biophysical Sciences, University of Houston, Houston, TX 77204-5934.

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between these changes by examining whether dietary supplementation with either *myo*-inositol or sorbinil, an aldose reductase inhibitor, was able to reverse or prevent the increased PIP_2 turnover in diabetic nerve. Two studies using *myo*-inositol–supplemented diets were performed. In the first, a high-sucrose diet was chosen because it was identical in composition to one previously shown to prevent decreased nerve conduction velocity and Na^+,K^+-ATPase activity. In the second, standard rat chow was supplemented with *myo*-inositol to eliminate possible extraneous effects of high carbohydrate intake.

MATERIALS AND METHODS

Materials

Sorbinil was supplied by Pfizer Central Research (Groton, CT). ^{32}P-inorganic phosphate was obtained from NEN-Dupont (Boston, MA). Sil-prep kits for derivatization of carbohydrates were from Alltech Associates (Houston, TX).

Induction of Diabetes and Preparation of Diets Supplemented With myo-Inositol or Sorbitol

Male Sprague-Dawley rats (200 to 230 g; Harlan Sprague-Dawley, Indianapolis, IN) were injected intravenously with streptozotocin (60 to 65 mg/kg body weight) dissolved in citrate buffer, pH 4.2. Streptozotocin-treated animals and age-matched normal rats were immediately placed on a designated diet (see below) and allowed food and water ad libitum. Dietary *myo*-inositol and sorbinil supplementation was performed as follows. In one experiment, 1% *myo*-inositol was incorporated into a synthetic high-sucrose diet[2] obtained from Nutritional Biochemicals (Cleveland, OH). This diet consisted of pellets containing 68% sucrose, 18% casein, 10% vegetable oil, 4% inorganic salts, and all necessary vitamins. In other experiments, diets were prepared by Ralston Purina (Richmond, IN). These diets consisted of Purina rat chow 5001 containing 1% *myo*-inositol or either high (0.05%) or low (0.02%) concentrations of sorbinil. Controls were fed the appropriate unsupplemented diet.

myo-Inositol Supplementation (experiments 1 and 2)

In these experiments, five groups of rats consisting of six animals each were used. The first group consisted of normal rats maintained on unsupplemented diets. The second group contained animals that were fed diets supplemented with *myo*-inositol. The third group consisted of diabetic rats that received unsupplemented diets. Rats in the fourth group were diabetic and were fed *myo*-inositol–supplemented diets throughout the experiment, whereas the fifth group of animals were also diabetic but were maintained on unsupplemented diets for the first 4 weeks and received the supplemented diet only during a subsequent 4-week period.

Sorbinil Supplementation (experiments 3 and 4)

To investigate the effects of dietary sorbinil, rats were divided into groups as described earlier. Normal rats were fed chow supplemented with 0.05% sorbinil, whereas diabetic animals were given chow containing 0.02% sorbinil in an effort to compensate for the larger food intake associated with diabetes.

An additional control experiment was performed to evaluate the effect of these differences in dietary sorbinil content. For this, three groups of normal animals were fed either the unsupplemented diet or diets containing 0.02% and 0.05% sorbinil for 8 weeks. For comparison, a group of diabetic animals also received the high-sorbinil diet and were killed after 5 weeks.

Analytical and Metabolic Procedures

Animals were killed by decapitation 8 weeks after streptozotocin injection. Blood was collected, and glucose level was determined as previously described.[18] Nerves were dissected and, except where indicated, desheathed. The tibial portion was used for analysis of carbohydrates by gas chromatography following derivatization to trimethylsilyl sugars.[19] In experiment 1, carbohydrates were analyzed using a standard 3% OV chromosorb W column (Alltech Associates, Deerfield, IL). In experiment 2, a 30-m DB1301 Megabore column (J & W Scientific, Folsom, CA) was used to obtain greater peak resolution. Determinations were performed using a Hewlett Packard 5830A gas chromatograph (Houston, TX) equipped with a flame ionization detector. The sample was injected at 180°C, and the column was eluted isothermally at 190°C.

^{32}P incorporation into phospholipids was examined using previously described procedures.[18,20] Briefly, segments of epineurium-free sciatic nerve were incubated at 37°C for 2 hours with $^{32}P_i$ in oxygenated Krebs-Ringer bicarbonate medium containing 5 mmol/L glucose. Lipids were extracted and separated by thin-layer chromatography to determine labeling of individual phospholipids.

RESULTS

Body Weight and Blood Glucose

Diabetic animals weighed much less than age-matched nondiabetic rats at the time of death in both the *myo*-inositol and sorbinil supplementation experiments (Table 1). Blood glucose levels were greater than 400 mg/dL within 48 hours after streptozotocin injection and were monitored at regular intervals thereafter. At the time of death, glucose concentrations for diabetic rats were greater than 430 mg/dL.

Table 1. Body Weights and Plasma Glucose Levels of Normal and Diabetic Rats

	Body Weight (g)		Blood Glucose (mg/100 mL)	
Conditions	Normal	Diabetic	Normal	Diabetic
Experiment 1				
No supplement	360 ± 18	263 ± 21	108 ± 13	645 ± 39
myo-Inositol supplement				
8 weeks	357 ± 14	250 ± 12	114 ± 8	506 ± 23
4 weeks	—	240 ± 14	—	436 ± 16
Experiment 2				
No supplement	410 ± 7	284 ± 11	115 ± 4	537 ± 10
myo-Inositol supplement				
4 weeks	—	302 ± 14	—	531 ± 19
8 weeks	407 ± 11	293 ± 15	126 ± 4	482 ± 13
Experiment 3				
No supplement	399 ± 9	282 ± 19	121 ± 2	540 ± 7
Sorbinil supplement				
4 weeks	—	297 ± 11	—	568 ± 5
8 weeks	414 ± 9	265 ± 15	121 ± 3	566 ± 7

NOTE. Data are the mean ± SEM from 6 rats for each condition.

Sorbinil Plasma Levels

Normal rats fed the 0.05% sorbinil–containing diet had plasma sorbinil levels after 8 weeks that were appreciably higher than those of diabetic rats fed the 0.02% sorbinil–containing diet for either 4 or 8 weeks (experiment 3; Table 2). Sorbinil concentrations in urine were 36.9 ± 4.0 μg/mL (mean ± SEM) for normal rats, as compared with 6.07 ± 0.76 and 7.16 ± 0.38 for diabetic rats fed sorbinil for 4 and 8 weeks, respectively. Normal animals excreted 73.6 ± 10.9 μg sorbinil (mean ± SEM) in 24 hours, whereas diabetic animals that received sorbinil for 4 and 8 weeks excreted 537 ± 100 and 785 ± 131 μg of the drug in the urine during the same period. These data indicate that marked dilution of sorbinil occurred in the copious urine produced by diabetic rats. The much greater output of urine by diabetic rats overshadowed their larger food intake and was probably mainly responsible for the lower plasma levels of sorbinil as compared with those in nondiabetic rats, notwithstanding the higher content of the aldose reductase inhibitor in the latter group of animals.

Sorbinil concentrations in normal rat plasma exhibited a dose-dependent effect when animals were fed either the high- or low-sorbinil diet (experiment 4; Table 2). On a high-sorbinil diet, normal and diabetic rats exhibited comparable plasma sorbinil levels.

Effects of myo-Inositol and Sorbinil Supplementation on Nerve Carbohydrate Content

In experiment 1, only *myo*-inositol and sorbitol were determined in tibial nerve from diabetic rats maintained on a high-sucrose diet identical to that used in a previous study[2] and in which approximately two thirds of the calories were derived from sucrose. The results showed a 50% decrease in the cyclitol and an increase in sorbitol ([in nanomoles per milligram wet weight nerve] normal [n = 4, *myo*-inositol 1.57 ± 0.21 and sorbitol <0.2; diabetic [n = 5], *myo*-inositol 0.80 ± 0.12 [$P < .05$ *v* normal by one-way ANOVA followed by Dunnett's test] and sorbitol 1.74 ± 0.38). Addition of 1% *myo*-inositol caused an elevation in *myo*-inositol content of nerve from normal rats (n = 6, 2.55 ± 0.30, $P < .01$ *v* normal), but was without a discernible effect on sorbitol levels. In nerves from diabetic rats fed the *myo*-inositol–supplemented diet, nerve *myo*-inositol levels were restored to normal, but there were no alterations in sorbitol content (after 4 weeks (n = 6), *myo*-inositol 1.73 ± 0.16 [$P < .01$ *v* untreated diabetic] and sorbitol 1.77 ± 0.16; after 8 weeks (n = 6), *myo*-inositol 1.48 ± 0.19 [$P < .01$ *v* untreated diabetic] and sorbitol 1.64 ± 0.22).

Table 2. Plasma Levels of Sorbinil (μg/mL) in Normal and Diabetic Rats

Dietary Sorbinil Content	Normal Rats		Diabetic Rats	
	4 Weeks	8 Weeks	4 Weeks	8 Weeks
Experiment 3				
0.02%	—	—	4.75 ± 0.22	4.43 ± 0.32
0.05%	—	8.04 ± 0.35	—	—
Experiment 4				
0.02%	3.69 ± 0.13	1.82 ± 0.19	—	—
0.05%	6.31 ± 0.52	5.94 ± 0.22	6.06 ± 0.89*	—

NOTE. Normal rats were fed a sorbinil-supplemented diet for 8 weeks. Diabetic rats were fed this diet for either 4 or 8 weeks as indicated. Data are expressed as the mean ± SEM for ≥6 rats in each condition.

*Sorbinil level was determined after 5 weeks of diabetes.

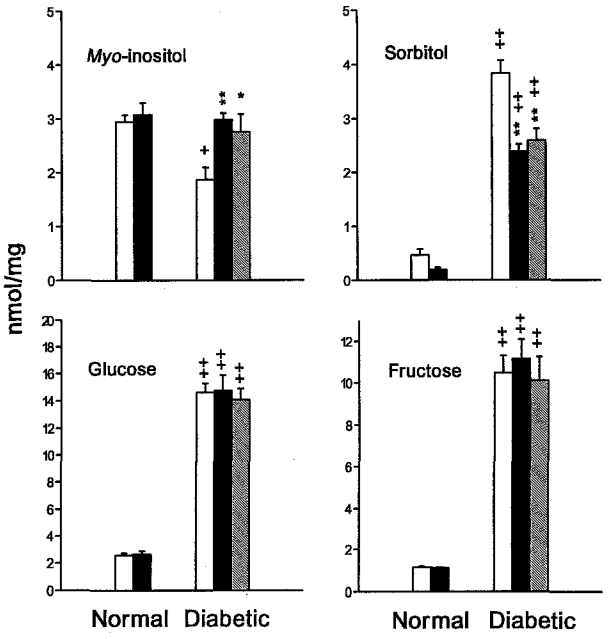


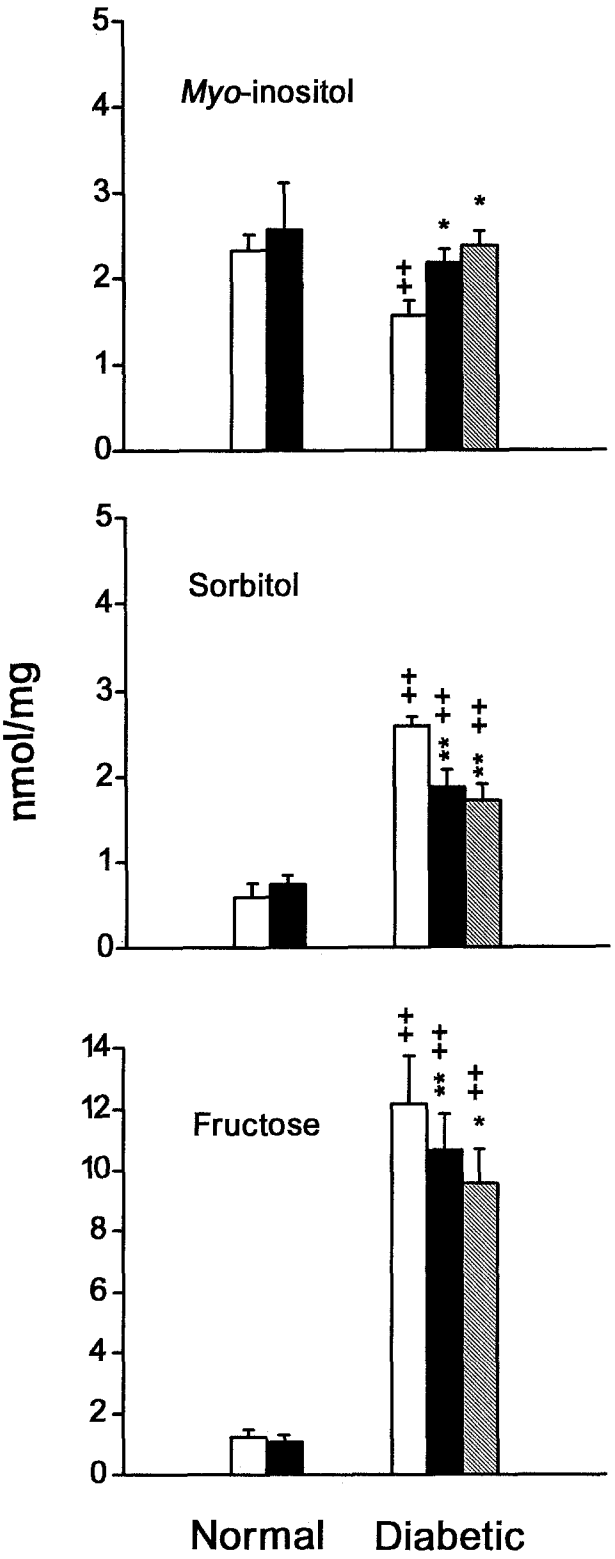
Fig 1. Fructose, sorbitol, glucose, and *myo*-inositol content (nmol/mg wet weight nerve) of tibial nerve from normal and streptozotocin-induced diabetic rats fed diets either with or without added *myo*-inositol. (□) Purina 5001 rat chow; (■) rat chow plus 1% *myo*-inositol for 8 weeks; (▨) rat chow plus 1% *myo*-inositol beginning after 4 weeks of diabetes. Each value represents the mean ± SEM for samples of 3 to 6 rats. Statistical significance between means was tested using one-way ANOVA followed by the Tukey-Kramer test. $^{+}P < .05$, $^{++}P < .01$: *v* normal animals fed standard rat chow diet. $^{*}P < .05$, $^{**}P < .01$: *v* untreated diabetic animals.

In experiment 2, a more complete analysis of carbohydrates was performed using a chromatographic column that produced sharper separation of individual peaks and hence greater sensitivity. Determinations of carbohydrates in nerves from diabetic rats fed the standard rat chow diet showed marked increases in glucose, sorbitol, and fructose concentrations and a 30% decrease in *myo*-inositol levels (Figs 1 and 2). For reasons that are unclear, absolute nerve *myo*-inositol and sorbitol contents were higher than in experiment 1. Inclusion of 1% *myo*-inositol in the chow diet of normal animals produced no change except for a decline in the content of sorbitol. Diabetic rats fed *myo*-inositol–supplemented chow exhibited a restoration of nerve *myo*-inositol to normal levels after both 4 and 8 weeks (Fig 1). Moreover, the elevated amounts of sorbitol in diabetic nerve declined 40% to 50%, although they were still well above control levels. However, fructose and glucose concentrations were unaffected.

Sorbinil-supplemented diets (experiment 3) had no effect on carbohydrate levels in nerves of nondiabetic animals, but in the case of diabetic rats they elicited a reduction of fructose and sorbitol levels and a correction of *myo*-inositol content to normal (Fig 2).

Effect of myo-Inositol Supplementation on Polyphosphoinositide Turnover

The effect of dietary *myo*-inositol on polyphosphoinositide turnover was examined in nerves from animals fed either the high-sucrose diet or the standard rat chow diet. Nerves from diabetic rats maintained on the high-sucrose diet without added *myo*-inositol exhibited markedly enhanced incorporation of ^{32}P into PIP_2 relative to controls (experiment 1; Table 3). Radioactivity in phosphatidylinositol-4-phosphate (PIP) was also significantly elevated, and that in phosphatidylinositol was slightly increased. When 1% *myo*-inositol was added to the high-sucrose diet, nerves from nondiabetic rats exhibited a trend toward lower isotope uptake into these phospholipids, but the effect was not statistically significant except for PIP (Table 3). Nerves from diabetic rats fed the *myo*-inositol–supplemented diet for either 4 or 8 weeks exhibited significantly less PIP_2 labeling as compared with nerves from untreated diabetic animals, but the incorporated radioactivity remained significantly elevated when compared with levels in either normal group. These treatments also reduced the uptake of isotope into PIP in diabetic nerve, although labeling of this lipid remained significantly above the values for nerves from normal animals fed the *myo*-inositol–supplemented diet. Labeling of phosphatidylinositol was decreased less than that for untreated diabetic rats after 8 weeks but not after 4 weeks. Neither diabetes nor dietary supplementation significantly altered the amount of radioactivity incorporated into either phosphatidylcholine or phosphatidylethanolamine (data not shown).



Because of the possibility that high sucrose intake might impose an abnormal carbohydrate load on the animals that could interfere with the effects of *myo*-inositol on phospholipid metabolism, another experiment was performed in which 1% *myo*-inositol was added to a standard rat chow diet (experiment 2; Fig 3). In contrast to the high-sucrose diet, inclusion of *myo*-inositol did not depress phosphoinositide labeling in normal rat nerve. Only PIP_2 labeling was increased in diabetic rats fed the standard rat chow diet. Administration of chow containing added *myo*-inositol for 8 weeks failed to produce correction of elevated polyphosphoinositide turnover in nerve from diabetic rats. Nerves from animals that had been fed the diet for 4 weeks showed what appeared to be partial reversal of increased labeling of these lipids (Fig 3). Once again, neither phosphatidylcholine nor phosphatidylethanolamine radioactivity were altered under any of the experimental conditions.

Effect of Sorbinil Supplementation on Polyphosphoinositide Turnover

As in the *myo*-inositol supplementation experiments, nerves from diabetic animals maintained on standard rat chow exhibited substantially increased PIP_2 turnover, whereas PIP and phosphatidylinositol metabolism was unchanged (Fig 4). Inclusion of 0.05% sorbinil in the chow

Fig 2. Fructose, sorbitol, and *myo*-inositol content (nmol/mg wet weight of nerve) of tibial nerve from normal and streptozotocin-induced diabetic rats fed diets either with or without added sorbinil. (□) Purina 5001 rat chow; (■) rat chow plus 0.05% sorbinil for 8 weeks in normal rats or 0.02% sorbinil for 8 weeks in diabetic rats; (▨) rat chow plus 0.02% sorbinil beginning after 4 weeks of diabetes. Each value represents the mean ± SEM for samples of 3 to 6 rats. Statistical significance between means was tested using one-way ANOVA followed by the Tukey-Kramer test. $^{+}P < .05$, $^{++}P < .01$: *v* normal animals fed a standard rat chow diet. $^{*}P < .05$, $^{**}P < .01$: *v* untreated diabetic animals.

Table 3. Effect of *myo*-Inositol–Supplemented High-Sucrose Diet on ^{32}P Incorporation Into Nerve Phosphoinositides (nmol ^{32}P incorporated/mmol lipid P)

Phosphoinositide	Normal	Normal + mI	Diabetic	Diabetic + mI (8 weeks)	Diabetic + mI (4 weeks)
PIP_2	293 ± 27	237 ± 28	511 ± 41†§	386 ± 29*§¶	392 ± 20*§‖
PIP	119 ± 12	85 ± 10*	181 ± 14†§	129 ± 11‡¶	143 ± 17§‖
PI	104 ± 7	86 ± 5	129 ± 7*§	103 ± 5‖	111 ± 8‡

NOTE. Data are the mean ± SEM from incubations of desheathed nerves from 5 to 6 rats: Statistical analysis was performed by one-way ANOVA followed by Dunnett's test.

Abbreviations: mI, *myo*-inositol; PI, phosphatidylinositol.

*$P < .05$, †$P < .01$: *v* normal animals fed an unsupplemented diet.

‡$P < .05$, §$P < .01$: *v* normal animals fed a *myo*-inositol–supplemented diet.

‖$P < .05$, ¶$P < .01$: *v* untreated diabetic animals.

diet depressed the quantity of isotope taken up into PIP_2 and PIP by 6% and 30%, respectively, in nerves of nondiabetic rats, and labeling of other phospholipids was also reduced, up to 44% in the case of phosphatidylcholine (data not shown). In contrast, when diabetic animals were fed a diet containing 0.02% sorbinil for either 4 or 8 weeks, ^{32}P incorporation into polyphosphoinositides was indistinguishable from isotope uptake into these lipids in normal nerve.

Effect of Variations in Dietary Sorbinil on Carbohydrate Content and Polyphosphoinositide Turnover in Normal Rats

As previously noted, normal and diabetic rats were fed diets in which the content of sorbinil was dissimilar, a procedure that resulted in higher sorbinil plasma levels in nondiabetic animals. This difference could complicate interpretation of the effects of sorbinil administration, and an additional experiment (experiment 4) was therefore performed to evaluate the impact of different amounts of dietary sorbinil on the results. Analysis of carbohydrates showed no significant difference in the levels of fructose, sorbitol, or *myo*-inositol in nerves of normal rats that had access to unsupplemented rat chow or to diets supplemented with either 0.02% or 0.05% sorbinil (data not shown). There was no measurable effect on the amount of isotope uptake into nerve phospholipids of normal animals fed the lesser amount of aldose reductase inhibitor, whereas 0.05% sorbinil caused slight to modest reduction in labeling. The decreases were 7% for PIP_2, 23% for PIP, and 18% for phosphatidylcholine (data not shown). We conclude that sorbinil at a sufficiently high level will depress phospholipid metabolism in normal rat nerve, albeit to a variable extent.

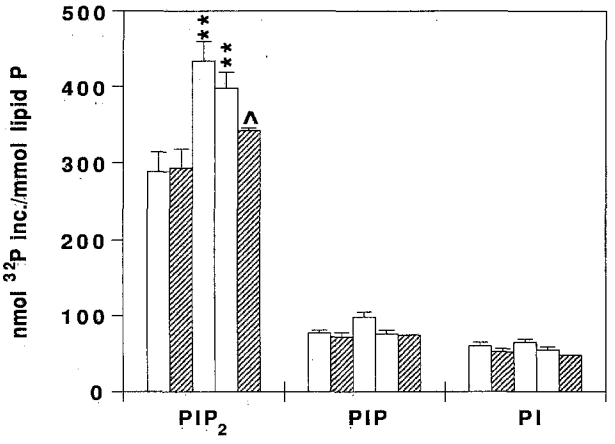


Fig 3. Effect of a standard laboratory chow diet supplemented with *myo*-inositol on the incorporation of $[^{32}P]P_i$ into phosphoinositides in nerve segments from normal and diabetic rats. Normal and diabetic rats were made diabetic and fed either standard rat chow or chow supplemented with 1% *myo*-inositol for the periods indicated. Nerves were incubated, and incorporation of radioactivity was measured. In each cluster, bars from left to right are as follows: bar 1, normal rats fed a standard diet for 8 weeks; bar 2, normal rats fed a *myo*-inositol–supplemented diet for 8 weeks; bar 3, diabetic rats fed a standard diet for 8 weeks; bar 4, diabetic rats fed a *myo*-inositol–supplemented diet for 8 weeks; and bar 5, rats fed a *myo*-inositol–supplemented diet for 4 weeks beginning 4 weeks after induction of diabetes. Values are the mean ± SEM for incubations of nerves from 6 animals. Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. **$P < .01$, *$P < .05$: *v* normal animals fed a standard rat chow diet. ^$P < .05$ *v* untreated diabetic animals.

Effect of Sorbinil on Nerve Phosphoinositide Metabolism In Vitro

The presence of sorbinil 10 μmol/L in the incubation medium did not significantly affect the quantity of ^{32}P incorporated into nerve phosphoinositides and failed to

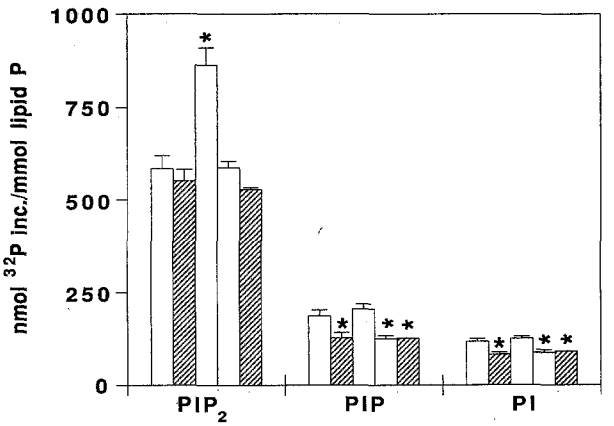


Fig 4. Effect of a standard rat chow diet supplemented with sorbinil on the incorporation $[^{32}P]P_i$ into phosphoinositides in nerve segments from normal and diabetic rats. The experiment was performed as described in Fig 3, and the order of bars in each cluster is the same as in Fig 3. Values are the mean ± SEM for incubations of nerves from 6 animals. *$P < .05$ *v* normal animals fed a standard rat chow diet, determined by one-way ANOVA followed by Dunnett's test.

blunt the enhanced isotope uptake into PIP_2 in diabetic nerve (data not shown).

DISCUSSION

The multiplicity and complexity of biochemical alterations reported to occur in experimental diabetic neuropathy have thus far precluded formulation of an hypothesis for the pathogenesis of the disease sufficiently comprehensive to account for and interrelate all of the documented changes. As one step toward this goal, we sought in this study to determine whether treatments of diabetic rats intended either to maintain the normal content or to restore the altered levels of nerve sorbitol, fructose, and *myo*-inositol to normal would prevent or correct the increased turnover of the monoesterified phosphate groups of PIP_2.

Administration of *myo*-inositol was effective both in restoring the depleted level of cyclitol and in preventing its loss in diabetic nerve, as reported by previous investigators.[3,4] Interestingly, when added to the standard rat chow diet but not to the high-sucrose diet, *myo*-inositol substantially reduced the increased sorbitol concentration. This decrease may be more apparent than real and may reflect experimental variability, because the level of sorbitol was considerably higher in nerves from diabetic rats in experiment 2 than in experiments 1 or 3. However, it has also been shown that aldose reductase can be induced upon exposure of cells to hypertonic extracellular fluid, thereby causing enhanced intracellular sorbitol formation as an osmoregulatory response.[25] In tissues and cells subjected to hyperglycemic conditions, this effect has been proposed to explain the accumulation of sorbitol and a consequent coordinate decrease in *myo*-inositol.[26,27] In this context, the decrease of sorbitol that accompanies the increase in *myo*-inositol in nerves from animals that received dietary *myo*-inositol supplementation may also reflect a compensatory effect associated with the osmoregulatory properties of these compounds. The failure of elevated fructose levels to change upon *myo*-inositol administration to diabetic rats suggests that this sugar may not contribute to these osmolyte responses.

As previously demonstrated, nerves from diabetic animals exhibited enhanced PIP_2 metabolism in all of the experiments. Diabetes also increased PIP metabolism when rats were fed a high-sucrose diet, but not when rats were fed a standard rat chow diet. The response of phosphatidylinositol to diabetes was also variable in that the labeling of this phospholipid was greater only in the experiment that used the high-sucrose diet. However, these differences are unlikely to be explained by variations in dietary composition, because in earlier studies in which animals were fed standard rat chow, increased labeling of PIP in diabetic nerve occurred in approximately half of the experiments, whereas that of phosphatidylinositol was rarely observed.[18,22,28] One explanation for these inconsistencies may be the varying severity of diabetes induced by streptozotocin.

Treatment of diabetic rats with *myo*-inositol, whether given as a supplement in a high-sucrose diet or added to standard rat chow, partially restored but failed to correct completely the abnormally high PIP_2 monoesterified phosphate group turnover in nerve. Thus, altered PIP_2 metabolism via the substrate cycle is influenced by factors other than prevailing levels of nerve *myo*-inositol. Previous studies have shown that in nerves from diabetic rats, incorporation of *myo*-inositol into phosphoinositides is reduced,[29,30] a finding that may reflect altered activity of the phosphoinositide cycle due to depletion of a discrete pool of the cyclitol[31] (and S. Abe, X. Zhu, and J. Eichberg, unpublished observations, February 1991).

Treatment of diabetic animals with sorbinil maintained or restored *myo*-inositol levels and decreased elevated sorbitol and fructose concentrations by approximately 30% and 20%, respectively. In previous studies, aldose reductase inhibitors have usually been given by gavage, but some investigators have added these agents to the diet at concentrations similar to those used here.[32,33] In some but not all of these studies, sorbitol levels have not returned to normal as a result of aldose reductase inhibitor treatment,[27,32] and fructose has also sometimes been reported to be less responsive than sorbitol to aldose reductase inhibitor administration.[32,34] These variable results may be due to an insufficient sustained systemic level of aldose reductase inhibitor due to differences in dose, route of administration, and half-life of the agent. Nonetheless, examination of data from virtually all studies concerning the effect of aldose reductase inhibitor treatment of diabetic animals reveals that regardless of the extent to which sorbitol concentration is decreased, nerve *myo*-inositol levels in diabetic animals are corrected to normal or near-normal levels.

Addition of sorbinil to the diet at a sufficiently high level (0.05%) was shown to depress ^{32}P incorporation into individual phospholipid classes in nerves from normal animals to varying extents. Phosphatidylcholine labeling was most affected, whereas uptake of isotope into PIP_2 was not significantly reduced. The mechanism responsible for this action of sorbinil remains to be elucidated, but indicates that this aldose reductase inhibitor may have more complex effects on nerve metabolism than hitherto appreciated. The ability of sorbinil to completely prevent or correct the enhanced turnover of monoesterified phosphate moieties of PIP_2 in diabetic nerve despite the presence of substantial residual sorbitol strongly suggests that the abnormal turnover of these groups is not closely associated with increased polyol pathway activity.

The factors that regulate the rapid cycling of PIP_2 phosphate groups in nerve are unknown, but could include both the activity of the enzymes involved and their accessibility to the substrate at sites in the myelin sheath. Measurements of activities of phosphatidylinositol 4-kinase and PIP_2 phosphomonoesterase have shown no substantial differences between these activities in normal and diabetic nerve, whereas PIP 5-kinase activity was marginally reduced in diabetic tissue.[35] Pulse-chase experiments have been interpreted to suggest that the actively renewed metabolic pool of PIP_2 phosphate groups comprises a small

portion of total myelin PIP_2, the bulk of which is metabolically relatively inert.[20] We have speculated that the locations of these postulated large and small metabolic compartments of PIP_2 are, respectively, in compact myelin and at sites adjacent to cytoplasm such as the paranodal loops or Schmidt-Lantermann incisures. It is possible that the diabetic state brings about subtle structural modifications in myelin such that the pool of rapidly cycling PIP_2 molecules is enlarged.

In summary, evidence is presented in this study that the enhanced metabolism of PIP_2 monoesterified phosphate moieties that occurs in diabetic nerve is not well correlated with either an elevated sorbitol level or a diminished *myo*-inositol concentration. These findings underscore complexities in the relationships among the biochemical alterations in nerve produced by experimental diabetes. Integration of these results into our knowledge concerning the pathogenesis of diabetic neuropathy must await greater understanding of the physiological importance of PIP_2 substrate cycling to nerve function.

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