Action of Sorbinil in Diabetic Peripheral Nerve

Relationship of Polyol (Sorbitol) Pathway Inhibition to a *myo*-Inositol-mediated Defect in Sodium-Potassium ATPase Activity

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SUMMARY

The small, but statistically significant, improvement in nerve conduction after treatment of diabetic patients with the aldose reductase inhibitor, sorbinil, suggests that increased polyol (sorbitol) pathway activity may contribute to diabetic nerve conduction slowing. Although classically viewed solely in terms of sorbitol-induced osmotic swelling, polyol pathway inhibition is now speculated to influence a concomitant *myo*-inositol-mediated alteration in nerve sodium-potassium ATPase activity in diabetic nerve. Therefore, we directly examined the effect of sorbinil treatment on sodium-potassium ATPase activity in crude homogenates of sciatic nerve from streptozotocin-diabetic and nondiabetic rats. We demonstrate that sorbinil treatment, which preserves normal nerve *myo*-inositol content, prevents the fall in nerve sodium-potassium ATPase activity that has been linked to conduction slowing in the diabetic rat. DIABETES *33*:712–716, August 1984.

Nerve conduction impairment in human diabetes is thought to reflect both chronic structural and reversible metabolic derangements in peripheral nerve;[1] however, the responsible metabolic factors remain unidentified.[2] One such possible metabolic defect is the increase in nerve polyol (sorbitol) pathway activity resulting from hyperglycemia, which leads to an accumulation of sorbitol and fructose in peripheral nerve. Treatment of diabetic patients with the polyol pathway inhibitor, sorbinil, is associated with small, but statistically significant, improvement in motor nerve conduction velocity (MNCV), suggesting that aldose reductase inhibition may influence a reversible component of nerve conduction impairment in diabetic subjects.[3] Although classically viewed only in terms of sorbitol-induced osmotic swelling,[4] the effects of polyol pathway inhibitors on diabetic peripheral nerve are probably much more complex,[5] involving not only polyol pathway intermediates but also concomitant alterations in nerve *myo*-inositol (MI) metabolism.[6–9]

Nerve MI is reduced in both human[10] and experimental diabetes as a consequence of hyperglycemia and insulin deficiency.[11] This metabolic alteration has been firmly implicated in nerve conduction slowing in the experimentally diabetic rat, in which treatment with insulin or oral MI supplementation,[11,12] or with aldose reductase inhibitors[7,8] prevents both the fall in nerve MI and nerve conduction velocity. (MI, a cyclic hexahydroxy hexanol that is found in high concentration in most mammalian tissues including nerve, is rapidly and reversibly incorporated into a labile class of phospholipids, the phosphoinositides, which are thought to have important membrane functions.[2])

Neural MI deficiency is thought to secondarily impair peripheral nerve membrane-bound sodium-potassium ATPase activity,[13] probably by a phosphoinositide mechanism,[14] leading sequentially to an increase in intraaxonal sodium concentration, a reduction in voltage-dependent sodium influx with depolarization, and a blunting of nerve impulse conduction.[11,15] This construct implies that sorbinil treatment, which reduces polyol pathway activity and prevents the fall in sciatic nerve MI in diabetic rats,[7,9] should also preserve normal nerve sodium-potassium ATPase activity. Therefore, we measured ouabain-inhibited and sodium-plus-potassium–stimulated ATPase activity in crude homogenates of sciatic nerve from untreated and sorbinil-treated streptozotocin (STZ)-diabetic and nondiabetic rats. We demonstrate that sorbinil treatment, which prevents the fall in nerve MI content, also prevents the 40%–45% reduction in nerve sodium-potassium ATPase activity that otherwise accompanies untreated STZ diabetes.[13] These studies suggest that the component of nerve conduction slowing reversed by sorbinil treatment in diabetics may reflect, in part, a MI-mediated sodium-potassium ATPase defect in peripheral nerve.

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Received for publication 3 November 1983.

MATERIALS AND METHODS

Animal model. Cesarean-delivered, barrier-sustained, male Wistar rats (initial weight 180–200 g) were maintained through-

TABLE 1
Effect of STZ diabetes and sorbinil treatment on rat body weight, plasma glucose, and *myo*-inositol (MI) concentration, and sciatic nerve MI content

Animal	N	Body weight* (g)	Plasma glucose* (mg/dl)	Plasma MI* (μM)	Sciatic nerve MI* (mmol/kg)
Nondiabetic	32	374 ± 6	165 ± 5	42.3 ± 2.9	2.79 ± 0.11
Nondiabetic sorbinil treated	8	369 ± 8	156 ± 5	35.2 ± 3.9	2.92 ± 0.13
					< 0.005
					NS
Diabetic	24	221 ± 9	589 ± 28	39.9 ± 2.9	2.26 ± 0.11
Diabetic sorbinil treated	18	228 ± 8	623 ± 25	43.7 ± 2.6	3.12 ± 0.14

*Values determined at the end of the 4-wk study period.

out the 4-wk study on a 0.011% MI (wt/wt) synthetic diet (Nutritional Biochemical Corporation, Cleveland, Ohio).[11–13] Diabetes, defined as nonfasting plasma glucose concentrations > 300 mg/dl, was induced with i.v. streptozotocin (STZ, Upjohn Company, Kalamazoo, Michigan), 60 mg/kg, as previously described.[13] Sorbinil (Pfizer Research, Pfizer Corporation, Groton, Connecticut) was suspended in distilled water (final concentration 4–5 mg/ml) as previously described[9] and administered daily by gavage at a dose of 20 mg/kg.

Tissue and plasma collection. At the conclusion of the 4-wk study, nonfasted animals were anesthetized with sodium pentobarbitol. Midthigh segments of left and right sciatic nerves were surgically removed, weighed, and processed either for enzymatic ATPase studies or for gas-liquid chromatographic determination of MI content as previously described in detail.[13]

Analytic techniques. Plasma glucose was determined in a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, California). Plasma and sciatic nerve MI were determined gas-liquid chromatographically in protein-free Somogyi filtrates of cardiac blood plasma or sciatic nerve homogenates as previously described.[11,12] Sodium-plus-potassium–stimulated and ouabain-inhibited ATPase activity was measured in freshly prepared crude homogenates of whole sciatic nerve as previously described in detail.[13] Briefly, nerve segments were minced and homogenized at 4°C in 2 ml of 0.2 M sucrose–0.02 M Tris-HCl, pH 7.5, using a Polytron homogenizer (Model PT 10-35, Brinkman Instruments, Inc., Westbury, New York). Five to 20 μl of homogenate were assayed enzymatically for ATPase activity in 1 ml of reaction mixture containing 100 mM NaCl, 10 mM KCl, 2.5 mM $MgCl_2$, 1 mM Tris-ATP, 1 mM tri(cyclohexylammonium) phosphoenolypyruvate, 30 mM imidazole-HCl buffer (pH 7.3), 0.15 mM NADH, 50 μg of lactate dehydrogenase, and 30 μg of pyruvate kinase.[16] After initial stabilization, composite ATPase activity was monitored spectrophotometrically as the oxidation of NADH. Sodium-plus-potassium–stimulated ATPase activity was computed by subtracting the reaction rate in an identical cuvette from which NaCl and KCl were omitted. Ouabain-inhibited ATPase activity was measured by comparing the linear reaction rates before and after the addition of 0.10 mM ouabain (final concentration). (Ouabain had no effect on the apparent rate of the reaction in the absence of both sodium and potassium ions.) After initial stabilization, composite, ouabain-inhibited, and sodium-plus-potassium–stimulated activities were linear with time for at least 45 min and proportional to protein concentration between 2.5 and 15 μg/ml.

All reagents were obtained from Sigma Chemical Co. (St. Louis, Missouri) and were of the highest available purity unless otherwise stated.

Statistics. Results are presented as mean ± SEM, and significance of difference was calculated by Student's *t* test.[17]

RESULTS

EFFECT OF SORBINIL ON STZ DIABETES AND PLASMA AND SCIATIC NERVE MI CONCENTRATION (TABLE 1)

The normal gain in body weight was blunted by diabetes to a similar degree in both sorbinil-treated and untreated rats

TABLE 2
Effect of STZ diabetes and sorbinil treatment on rat sciatic nerve ATPase activity

Animal	N	ATPase activity (μmol/g wet wt/h)* Composite	Ouabain-inhibited	Sodium + potassium-stimulated	Non-ouabain–inhibited
Nondiabetic	32	431 ± 10	103.8 ± 3.9	104.3 ± 4.4	327 ± 8
		NS	NS	NS	NS
Nondiabetic sorbinil treated	8	455 ± 27	97.6 ± 6.2	97.6 ± 6.2	357 ± 25
		< 0.001	< 0.001	< 0.001	< 0.001
		< 0.010	NS	NS	< 0.025
Diabetic	24	321 ± 12	58.2 ± 2.6	56.8 ± 2.9	263 ± 17
		< 0.025	< 0.001	< 0.001	NS
Diabetic sorbinil treated	18	374 ± 18	95.8 ± 5.0	95.7 ± 5.0	278 ± 17

*Values determined at the end of the 4-wk study period.

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(column 1). Sorbinil treatment did not alter nonfasting plasma glucose in either diabetic or nondiabetic rats (column 2). Plasma MI was unaltered by experimental diabetes or sorbinil treatment either separately or in concert (column 3). Thus, body weight, hyperglycemia, and plasma MI were similar in untreated and sorbinil-treated diabetic rats.

Sciatic nerve MI content (column 4) was unaffected by sorbinil treatment in the nondiabetic rats. Nerve MI was significantly reduced by diabetes, but this decrease was completely prevented by sorbinil. Thus, sorbinil administration prevented the abnormality in nerve MI metabolism consistently associated with acute STZ diabetes in the rat.[7-9,11-13]

EFFECT OF SORBINIL ON SCIATIC NERVE SODIUM-POTASSIUM ATPase ACTIVITY (TABLE 2, FIGURE 1)

Composite ATPase activity (Table 2, column 1). Sorbinil administration did not affect composite ATPase activity, expressed per gram wet weight of nerve, in crude homogenates of sciatic nerve from nondiabetic rats. Sciatic nerve composite ATPase activity was reduced 25% in untreated diabetic rats. Sorbinil treatment significantly raised (by 17%), but did not normalize, diabetic sciatic nerve composite ATPase activity ($P < 0.025$, line 3 versus line 4).

Ouabain-inhibited ATPase activity (Table 2, column 2). Composite sciatic nerve ATPase activity was inhibited similarly by ouabain (0.1 mM) in sorbinil-treated and untreated nondiabetic rats (by 21% and 24%, respectively); thus the absolute magnitude of the ouabain-inhibited ATPase fraction was not altered by sorbinil treatment in nondiabetic rats (compare lines 1 and 2). The ouabain-inhibited ATPase component was diminished by 44% in untreated STZ-diabetic rats (line 3). Sorbinil treatment raised diabetic sciatic nerve ouabain-inhibited ATPase activity by 65%, so that it was no longer significantly smaller than that of either treated or untreated nondiabetic rats (line 4).

Sodium-plus-potassium–stimulated ATPase activity (Table 2, column 3; Figure 1). The absence of sodium and potassium ions inhibited composite ATPase activity in sciatic nerve homogenates to the same degree as did the presence

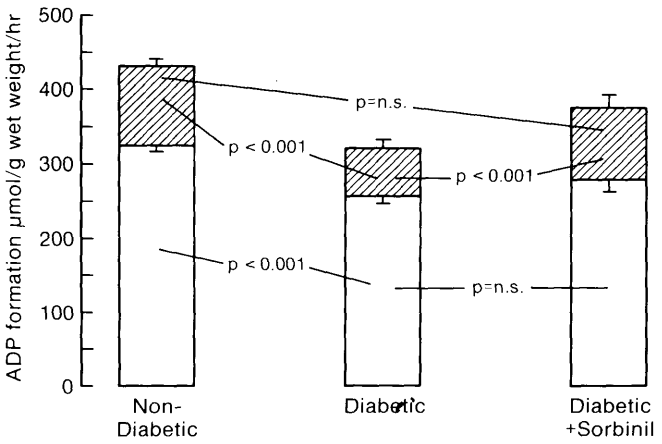

FIGURE 1. Effect of experimental diabetes and sorbinil treatment on composite and sodium-plus-potassium–stimulated ATPase activity in rat sciatic nerve. Composite ATPase activity is represented by the height of the bar. The cross-hatched portion of each bar represents the sodium-plus-potassium–stimulated component, while the open portion of each bar represents the non-sodium-plus-potassium–stimulated fraction.

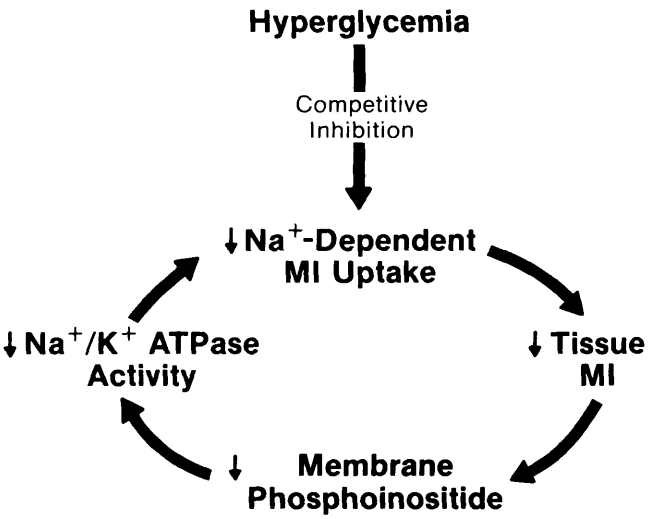

FIGURE 2. Proposed interaction of hyperglycemia, sodium-dependent *myo*-inositol (MI) uptake, inositol phospholipid metabolism, and sodium-potassium ATPase activity in the pathogenesis of diabetic neuropathy.[6,28]

of 0.1 mM ouabain in each experimental condition. Thus, ouabain-inhibited ATPase activity and sodium-plus-potassium–stimulated ATPase activity were of similar magnitude in each experimental group, and were similarly affected by experimental diabetes and sorbinil treatment (compare columns 2 and 3). Sorbinil did not affect nondiabetic sodium-plus-potassium–stimulated ATPase activity. Experimental diabetes reduced sodium-plus-potassium–stimulated ATPase activity by 46%, and sorbinil treatment raised diabetic sodium-plus-potassium–stimulated ATPase activity by 68%, so that it no longer differed significantly from sorbinil-treated or untreated nondiabetic rats (Figure 1). Thus, both ouabain inhibition and sodium-plus-potassium stimulation defined the same ATPase activity in sciatic nerve homogenates, i.e., the sodium-potassium ATPase.

Non-ouabain–inhibited ATPase activity (Table 2, column 4). Residual ATPase activity in the presence of ouabain was significantly lowered by experimental diabetes, but was unaffected by sorbinil in either diabetic or nondiabetic nerve. Similar results were obtained in non-sodium-plus-potassium–stimulated ATPase activity (Figure 1). Therefore, increased composite ATPase activity in sorbinil-treated diabetic nerve specifically reflected the rise in the sodium-potassium ATPase component.

DISCUSSION

Sorbinil administration increases MNCV in both diabetic rats and humans.[3,7] In the acutely diabetic rat, nerve conduction slowing is unaccompanied by significant structural alteration and is completely and rapidly reversible by metabolic treatment; it is, therefore, thought to be metabolically mediated.[2,6,18,19] On the other hand, rapidly reversible nerve conduction slowing is less evident in the chronically diabetic human,[20-24] presumably due to poorly reversible alterations in peripheral nerve structure.[1] However, the metabolic factors underlying reversible conduction slowing in the diabetic rat and human, and those responsible for structural changes in human diabetic nerves, are generally assumed to be

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closely related.[2] Therefore, neurophysiologic responses to metabolic agents, such as sorbinil, in both diabetic rats and patients help define pathogenetic elements of diabetic neuropathy.[3]

Nerve conduction impairment in the diabetic rat is attributed to a MI-related defect in neural sodium-potassium ATPase.[6,13,15] Aldose reductase inhibitors, including sorbinil, prevent the fall in diabetic nerve MI content[7-9] that is thought to underlie the sodium-potassium ATPase defect;[13] the present studies directly confirm that sorbinil administration prevents the concomitant fall in sciatic nerve sodium-potassium ATPase activity as well. (Peripheral nerve is a heterogeneous tissue comprised of axons, Schwann cells, the perineurial epithelium, and endo- and epineurial connective, vascular, and adipose tissue, all of which may posess sodium-potassium ATPase activity. Furthermore, enzymatic activities measured in tissue homogenates may or may not reflect the true activity of those enzymes within intact cells. However, the electrophysiologic observations cited above suggest that variation in whole nerve-homogenate, sodium-potassium ATPase activity parallels that of the cellular elements responsible for nerve impulse conduction in vivo.) In both the diabetic human and rat, nerve MI content is reduced, and sorbinil enhances MNCV.[3,7,10] The action of sorbinil on nerve conduction in the diabetic human,[3] thus, most likely reflects an effect on nerve MI content and sodium-potassium ATPase activity.

The sodium-potassium ATPase not only generates the membrane sodium potential necessary for nerve impulse conduction,[25] but also energizes such vital cell functions as the active uptake of metabolic substrates, and the regulation of intracellular pH, hydration, and calcium concentration;[26] therefore, a MI-related alteration in nerve sodium-potassium ATPase activity may mediate not only the acute, but also the more indolent, consequences of hyperglycemia on human nerve metabolism, function, and perhaps structure.

The mechanism by which aldose reductase inhibitors prevent the reduction in diabetic nerve MI content is not understood. However, nerve MI content and sodium-potassium ATPase function are intertwined in a complex, self-reinforcing cycle of sodium-gradient–dependent MI uptake (which is competitively inhibited by hyperglycemic concentrations of glucose[27]), altered nerve phosphoinositide metabolism,[14] and impaired phospholipid-dependent, membrane-bound, sodium-potassium ATPase activity[13] (see Figure 2). The molecular mechanisms linking free and lipid-bound *myo*-inositol with sodium-potassium ATPase activity in peripheral nerve remain to be clarified. However, inositol-containing phospholipids have been identified as a possible endogenous activator for rat renal sodium-potassium ATPase.[29] Sorbinil administration in the experimentally diabetic rat prevents the fall in both nerve MI content[7,9] and sodium-potassium ATPase activity; hence, it interrupts the postulated pathogenetic cycle illustrated in Figure 2. However, neither the locus of action of sorbinil within this cycle, nor its relationship to the drug's specific action as an aldose reductase inhibitor are presently known;[9,30] both questions are currently under investigation in this and other[8,30] laboratories.

In summary, sorbinil and, presumably, other polyol pathway inhibitors[8] prevent nerve conduction slowing in the diabetic rat by correcting underlying defects in MI and MI-phospholipid metabolism that secondarily impair sodium-potassium ATPase function. Similarities in the action of sorbinil in diabetic rats[7] and humans[3] suggest that analogous metabolic defects may be involved in reversible nerve conduction slowing in human diabetic subjects.[3]

ACKNOWLEDGMENTS

The authors wish to express their gratitude to Lorraine Weber, Jean Kim, and Carol Korbanic for expert technical assistance; to Dr. Michael Peterson, Pfizer Central Research, Groton, Connecticut, for providing the sorbinil used in these studies; and to Dr. Jan Ulbrecht for critically reviewing the manuscript.

These studies were supported in part by USPHS research grant #RO1-AM2982, Juvenile Diabetes Foundation research grant 82R231, and the Harry Soffer Memorial Research Fund.

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