Improve of Nerve Conduction Velocity in Mutant Diabetic Mice by Aldose Reductase Inhibitor without Affecting Nerve myo-Inositol Content

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The sciatic motor nerve conduction velocity of mutant diabetic C57BL/Ks mice was significantly improved from 30.0 ± 1.4 to 38.0 ± 4.6 m/s by treatment with the aldose reductase inhibitor 1-[β-naphthyl]sulfonfonyl]hydantoin (30 mg/kg/d) for 2 weeks. The treatment, however, did not cause any significant change in myo-inositol concentration in the sciatic nerve. The results indicate that the ameliorating effect of the aldose reductase inhibitor on nerve conduction velocity in mutant diabetic mice is not due to alteration of myo-inositol content in the nerve.

Keywords: aldose reductase; aldose reductase inhibitor; nerve conduction velocity; sorbitol; myo-inositol; mutant diabetic mice

Hyperglycemia-induced alterations in the metabolism of sorbitol and myo-inositol have been implicated in diabetic complications such as neuropathy, retinopathy, nephropathy, and cataract.1) The enzyme aldose reductase, which constitutes the sorbitol pathway together with sorbitol dehydrogenase, and is found in the tissues that exhibit diabetic complications, is thought to play a critical role in such metabolic alterations. Aldose reductase inhibitors that prevent sorbitol accumulation in nerves and ameliorate diabetic neuropathy characterized by decreased motor nerve conduction velocity (MNCV) were shown to block nerve myo-inositol depletion as well,2) although the mechanism is not yet known. Recent studies indicate that virtually all the known effects of aldose reductase inhibitors on the function of peripheral nerves in diabetic animals, and possibly in diabetic patients, may be explained by prevention of myo-inositol depletion in the nerve and consequent restoration of Na-K ATPase activity.3) The aim of the present study was to test whether an aldose reductase inhibitor, 1-[β-naphthyl]sulfonfonyl]hydantoin (β-NSH), affects MNCV and/or nerve myo-inositol content in mutant diabetic mice of the C57BL/Ks strain, which are reported to have a nerve myo-inositol content comparable to that of nondiabetic mice.4,5)

Materials and Methods

Animals: Mice used in this experiment were of either sex and of the C57BL/Ks strain (from Jackson Laboratory, Bar Harbor, ME, U.S.A.), which is characterized by an autosomal recessive gene (db). The homozygote (db/db) mice, which show full penetrance of the diabetic syndrome, were used as the experimental animals. The age-matched littermates with heterozygote (db/+ ) nonpenetrant genotype were used as the control animals.

Assay of Blood Glucose: Blood glucose levels of mice at the age of 13—14 weeks were determined after 4 h of fasting, using a glucose oxidase-peroxidase method (GOD-Perid Test: Boehringer, Mannheim, Federal Republic of Germany).

Administration of Drug: Diabetic mice (db/db) at the age of 13—14 weeks were divided into two groups: one group received for 14 d an oral administration of β-NSH (30 mg/kg/d), which was prepared as described previously6) and suspended in 50% gum arabic, and the other group was treated with the vehicle (50% gum arabic) for the same period of time. The vehicle was also given to nondiabetic control mice (db/+).

Measurement of Motor Nerve Conduction Velocity: Sciatic MNCV was measured 14 d following initiation of treatment. The measurement of sciatic MNCV was performed under ether anesthesia in an air-conditioned room (24—25 °C) by a modification of the method of Robertson and Sina.7) The sciatic nerve was stimulated with a needle electrode at the sciatic notch and the tibial nerve posterior to the ankle. Supramaximal 0.1 ms stimuli were delivered at 1 Hz. The action potentials were recorded from the muscle of the first interosseous space with a Nihon Kohden electromyograph, MEM-3202 (Nihon Kohden Corp., Tokyo, Japan), and the latencies were measured from a storage oscilloscope. The MNCV was calculated by dividing the distance (25 mm) between the two stimulation points by the difference in latency.

Assay of Sorbitol and myo-Inositol in Sciatic Nerve: Just after the measurement of MNCV, the mice were killed by decapitation under ether anesthesia, and both sciatic nerves were removed and stored at −80 °C for later determination of polysol contents. Sciatic nerve sorbitol and myo-inositol concentrations were determined by our method using high-performance liquid chromatography (HPLC).8) Briefly, polysols in lyophilized extracts were reacted with phenylisocyanate to give phenylcarbamoyl derivatives and then separated on a reverse-phase column packed with C18-bonded silica gel.

Statistics: All results are expressed as mean ± S.D. Statistical analysis was performed by the use of Student’s t test with the level of significance set at p < 0.05.

Results

The homozygote (db/db) mice developed hyperglycemia, obesity, polyuria, and glycosuria at the age of 5—6 weeks, whereas such pathological features were not observed even at the age of 14 weeks in the heterozygote (db/+ ) mice. Fasting blood glucose levels of homozygote and heterozygote mice at the age of 13—14 weeks were 22.4 ± 6.6 (n = 10) and 7.8 ± 0.6 (n = 10) mmol/l, respectively. Oral administration of β-NSH (30 mg/kg/d) or vehicle to diabetic and nondiabetic mice for 2 weeks did not cause any significant change in the blood glucose level.

The results of MNCV measurements are shown in Table I. Sciatic MNCV of diabetic mice was significantly improved from 30.0 ± 1.4 to 38.0 ± 4.6 m/s by treatment with

| Table I. Motor Nerve Conduction Velocity before and after Treatment with β-NSH or Vehicle |
|-----------------|-----------------|-----------------|-----------------|
|                  | Mice            | Motor nerve conduction velocity (m/s) | Before treatment | After treatment |
| Vehicle-treated  | 10              | 46.4 ± 3.9       | 46.2 ± 6.3      |
| nondiabetic      |                 |                 |                 |
| Vehicle-treated  | 10              | 30.1 ± 2.0       | 30.3 ± 2.9a     |
| diabetic         |                 |                 |                 |
| β-NSH-treated    | 10              | 30.0 ± 1.4       | 38.0 ± 4.6b,c   |
| nondiabetic      |                 |                 |                 |

a) Significantly different from vehicle-treated nondiabetic mice (p < 0.001).
b) Significantly different from vehicle-treated diabetic mice (p < 0.001).
c) Significantly different from vehicle-treated nondiabetic mice (p < 0.01).

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TABLE II. Polyol Contents in the Sciatic Nerve of Diabetic and Nondiabetic Mice

<table>
<thead>
<tr>
<th>Mice</th>
<th>n</th>
<th>myo-Inositol (μmol/g wet tissue)</th>
<th>Sorbitol (μmol/g wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle-treated nondiabetic</td>
<td>5</td>
<td>2.68 ± 0.29</td>
<td>0.066 ± 0.019</td>
</tr>
<tr>
<td>Vehicle-treated diabetic</td>
<td>5</td>
<td>2.69 ± 0.23</td>
<td>0.105 ± 0.029</td>
</tr>
<tr>
<td>β-NSH-treated diabetic</td>
<td>5</td>
<td>2.53 ± 0.12</td>
<td>0.070 ± 0.011</td>
</tr>
</tbody>
</table>

a) Significantly different from vehicle-treated nondiabetic mice (p < 0.05).
b) Significantly different from vehicle-treated diabetic mice (p < 0.05).

β-NSH (30 mg/kg/d) for 2 weeks. On the other hand, treatment with vehicle alone did not affect the MNCV values in the diabetic and nondiabetic mice. The MNCV (38.0 ± 4.6 m/s) in the diabetic mice treated with β-NSH was significantly higher than that (30.3 ± 2.9 m/s) in the vehicle-treated diabetic mice. There was no difference between males and females in MNCV in the diabetic mice treated with β-NSH: 38.6 ± 5.0 m/s (n = 5) for males vs. 37.4 ± 4.7 m/s (n = 5) for females.

Sorbitol and myo-inositol concentrations in the sciatic nerve of diabetic and nondiabetic mice are shown in Table II. Sorbitol levels in all groups were markedly low compared with myo-inositol levels. Nerve myo-inositol concentrations in the three groups were not significantly different.

Discussion

β-NSH is a potent aldose reductase inhibitor which was developed by us. Oral administration of β-NSH at 50 mg/kg/d for 12 d to streptozotocin-induced diabetic rats inhibited sorbitol accumulation in the sciatic nerve completely and prevented the fall in nerve myo-inositol content. Moreover, such β-NSH treatment significantly improved MNCV in streptozotocin-induced diabetic rats. Genetically diabetic mice of the C57BL/Ks strain were reported to lack sorbitol and fructose accumulation in peripheral nerves. Very recently, however, galactose feeding of diabetic and nondiabetic C57BL/Ks mice was found to result in marked accumulations of dulcitol in nerves, indicating the presence of polyol-forming activity (most probably aldose reductase) in mouse nerve. These reports suggest that sorbitol is metabolized rapidly to fructose by sorbitol dehydrogenase and fructose disappears by diffusion and/or rapid further metabolism, whereas dulcitol is not accessible to metabolism or diffusion. Our data also suggest that aldose reductase is present in the sciatic nerve of C57BL/Ks mice, since the nerve sorbitol content in the diabetic mice was significantly higher than that in the nondiabetic mice, but was returned to the level in nondiabetes by the treatment with β-NSH (Table II). Nevertheless, it does not appear that such small differences in nerve sorbitol level among the three groups would account for the variation in MNCV.

The present study showed that sciatic MNCV in the diabetic mice is significantly slower than that in the nondiabetic control mice (Table I), and that the sciatic nerve myo-inositol level does not differ between the two (Table II) in accordance with previous reports. The literature contains conflicting data on MNCV in C57BL/Ks mice. Robertson and Sima found markedly slow MNCV in diabetic mice relative to nondiabetic mice; but, on the other hand, Whiteley and Tomlinson detected no deficit in MNCV in diabetic mice. The reasons for such discrepancies are not clear but may be due to differences in methodology for determining MNCV. Housing, age, or other differences may also be contributing factors.

We wish to emphasize that sciatic MNCV in the diabetic mice treated with an aldose reductase inhibitor, β-NSH, was significantly higher than that in the vehicle-treated diabetic mice (Table I), whereas sciatic nerve myo-inositol levels in the two groups were not different from each other (Table II). These findings indicate that improvement of MNCV in diabetic mice by β-NSH treatment is not associated with normalization of nerve myo-inositol content. This leads to the view that in C57BL/Ks mice, β-NSH has pharmacological activities other than prevention of nerve myo-inositol depletion. One possible explanation is that the improvement of MNCV by β-NSH is due to inhibition of the metabolic flux of glucose through the sorbitol pathway, since a recent study suggested that diabetic complications may be related to an increased flux through the sorbitol pathway rather than accumulations of sorbitol and fructose per se. Aldose reductase inhibitors were shown to stimulate Na,K-ATPase activity by interacting directly with the enzyme. This is unlikely to be the case in C57BL/Ks mice, however, since it was reported that there is no difference in sciatic nerve Na,K-ATPase activity between diabetic and nondiabetic mice.

In conclusion, the present study indicates that the aldose reductase inhibitor β-NSH improves nerve conduction velocity in mutant diabetic mice without affecting nerve myo-inositol content.

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