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Myo-Inositol and Sorbitol Metabolism in Relation to Peripheral Nerve Function in Experimental Diabetes in the Rat: The Effect of Aldose Reductase Inhibition

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Summary. A possible relationship between increased sorbitol concentration and decreased myo-inositol concentration in peripheral nerves of diabetic rats has been examined. To this end, sorbinil, an aldose reductase inhibitor, was used either to prevent or reverse elevation of nerve sorbitol concentration in diabetic rats. Sorbinil treatment at 20 mg · kg^{-1} · day^{-1} prevented elevation of nerve sorbitol levels in early diabetes and reduced sorbitol concentration from 2.38 to 0.51 μmol/g in rats diabetic for 10 weeks. This treatment reduced the increase in nerve fructose concentration and prevented the reduced myo-inositol concentration found in diabetic rat nerve (control 3.63, diabetic 2.40, diabetic/sorbinil, 3.56 μmol/g). Sorbinil treatment did not prevent a significant slowing of motor-nerve conduction velocity at 10 weeks although treatment reduced the extent of slowing. Sorbinil treatment at 25 mg · kg^{-1} · day^{-1} reduced elevated sorbitol and fructose concentrations in diabetic rat nerve and normalised myo-inositol concentration. Myo-Inositol treatment at 650 mg · kg^{-1} · day^{-1} did not affect the elevated concentrations of sorbitol, fructose or glucose in peripheral nerves of diabetic rats, but it did restore reduced myo-inositol concentration. Both sorbinil and myo-inositol treatment partially reversed the slowing of motor-nerve conduction velocity in diabetic rats. These results are discussed in relation to the involvement of sorbitol and myo-inositol metabolism in the aetiology of diabetic neuropathy.

Key words: Myo-Inositol, sorbitol, motor nerve conduction velocity, aldose reductase, sorbinil.

Induction of experimental diabetes in the rat with alloxan or streptozotocin results in a well-characterized slowing of motor-nerve conduction velocity (MNCV) [1–5], mixed nerve conduction velocity [6] and a more recently demonstrated fall in sensory-nerve conduction velocity [7, 8]. Structural alterations also appear to be present and take the form of axonal degeneration [9–11], with possibly demyelination/remyelination [11] and myelin reduction [12].

It is now considered that early metabolic abnormalities, rather than vascular changes, cause diabetic neuropathy [13]. Peripheral nerve glucose concentration is markedly elevated following induction of diabetes and this leads to increased sorbitol levels [14, 15] via the action of the enzyme aldose reductase [15]. This increase has led to the sorbitol theory of diabetic neuropathy. Since sorbitol is not freely diffusible, its accumulation in compartments within peripheral nerve may lead to osmotic changes, cell damage and hence slowed nerve conduction velocity [15]. Accompanying these changes, although not necessarily connected with them, is a reduction in peripheral nerve free myo-inositol concentration [2, 14] with resultant altered metabolism of the phosphoinositides [8, 16–18] which are thought to be involved in conduction of the nerve impulse [19].

To date, no study has attempted to correlate increased polyol pathway activity in peripheral nerve during experimental diabetes with reduced free myo-inositol concentration. In recent years, increasing interest has been focussed on the potential to reduce nerve sorbitol concentrations of a group of compounds which inhibit aldose reductase. This study describes the use of such a compound, CP-45,634 (+)6-fluoro-spiro[chroman-4,4′-imidazolidine]-2′,5′-dione) ('sorbinil', Pfizer, Sandwich, Kent, UK) to reduce elevated sorbitol concentrations in streptozotocin-diabetic rat nerve. The consequent changes in myo-inositol concentration are described. In view of the implications this may have for nerve function in diabetes, serial motor-nerve conduction velocities were determined also in the same animals treated with sorbinil. In addition, a comparative study of the effect of either sorbinil or myo-inositol treatment on MNCV is described, since dietary myo-inositol supplementation has previously been shown to improve

MNCV in acutely diabetic rats [2]. A short preliminary account of some of the present work has been published [20].

Materials and Methods

Male Wistar rats weighing 270–320 g were from the Nottingham University Joint Animal Breeding Unit, Sutton Bonington, Leicestershire, UK and were fed standard laboratory diet ad libitum (Diet 41B, Heygate & Sons, Northampton, UK; myo-inositol content 0.022%).

Induction of diabetes was achieved by a single IP injection of streptozotocin (Sigma, Poole, Dorset, UK: dosage given in details of trials below) dissolved in 50 mmol/l sodium citrate buffer (pH 4.5) immediately before injection. Only those animals whose serum glucose concentration 2 days after injection exceeded 16 mmol/l were included in the trials.

Trial 1

This trial was designed to prevent the rise in nerve sorbitol concentrations in diabetic rats by the administration of sorbinil and to correlate this with myo-inositol concentration and MNCV.

MNCV was measured in three groups of age-matched animals. Two groups were injected with streptozotocin (50 mg/kg, IP); one group continued as diabetic controls and the second group received sorbinil by gastric intubation once daily ($20\ mg \cdot kg^{-1} \cdot day^{-1}$). Injection of streptozotocin and commencement of sorbinil treatment were performed on the day of the initial MNCV recording. Further MNCV measurements were made at 2, 4 and 10 weeks after the induction of diabetes. Sciatic nerve concentrations of sorbitol, fructose, glucose and myo-inositol were determined in groups of rats at 2 weeks and in the remainder at 10 weeks.

Trial 2

The aim of this trial was to assess the potential of sorbinil or myo-inositol treatment to reverse biochemical changes and slowed MNCV in diabetic rats. MNCV was measured in 80 age-matched rats. Sixty rats were injected with streptozotocin (35 mg/kg, IP), of which 34 developed diabetes of sufficient severity to be included in the trial. Six weeks later, MNCV was measured in the control and diabetic groups. The diabetic group was then divided into three sub-groups: one subgroup acted as diabetic controls, another received sorbinil by gastric intubation ($25\ mg \cdot kg^{-1} \cdot day^{-1}$) and the third sub-group received myo-inositol by gastric intubation ($650\ mg \cdot kg^{-1} \cdot day^{-1}$; 10% w/v in water). The myo-inositol dose was designed to administer an amount equivalent to that ingested from a myo-inositol supplemented diet which has previously been recommended [2]. MNCV was measured 3, 6 and 9 weeks following the initiation of treatment. Sciatic nerve sorbitol, fructose, glucose and myo-inositol concentrations were determined 9 weeks after commencement of treatment.

Measurement of MNCV

MNCV was measured by the method of Sharma and Thomas [4]. Rats were anaesthetized with sodium pentobarbitone ('Sagatal', May & Baker, Dagenham, Essex, UK: 60 mg/kg, IP). Fine needle electrodes were inserted adjacent to the sciatic nerve at the sciatic notch and then at the Achilles tendon. In each case the stimulating electrode was connected across the output of a Grass S88 stimulator (Grass Instruments, Stag Instruments, Henley-on-Thames, UK) with a reference electrode placed subcutaneously in the flank. Electromyograms (EMG) were recorded from the second interosseous muscle of the same limb and displayed on a Tektronix 5103 N storage oscilloscope (Tektronix UK, Harpenden, Herts, UK). Several EMG were obtained by stimulation (supramaximal voltage; 1 ms pulses) at the Achilles tendon followed by a second group from stimulation at the sciatic notch. The temporal separation of the two groups of EMG was measured. The length of nerve separating the two stimulation points was measured with dividers and was used to calculate MNCV. This procedure was performed at a constant rectal temperature of 37 °C with the rats on a heated pad in a room maintained at 25 °C.

Analyses

For the determination of sugar and sugar alcohol concentrations, sciatic nerves were removed, following MNCV recording, from the level of the sciatic notch and extending approximately 3.5 cm distally. The epineurium was removed rapidly, the nerve weighed and immediately frozen in liquid nitrogen. Nerves were stored at −80 °C until analysis.

To determine fructose, glucose and myo-inostitol concentrations, nerves were placed in boiling distilled water for 3 min followed by homogenisation and deproteinisation with $ZnSO_4$ and $Ba(OH)_2$ as described by Greene et al. [2]. Following centrifugation, aliquots were lyophylised before analysis by gas chromatography as described previously [14] with one modification. The shaking time for the formation of trimethylsilyl derivatives was increased from 4 to 16 h. This method proved to be suitable for the determination of glucose and fructose as well as the heat-stable myo-inositol. Recovery of high concentrations of glucose and fructose added to control rat nerve extractions was 95 and 72%, respectively and this did not alter significantly in extracts which were not boiled. Boiling high concentrations of standard glucose or fructose also did not result in temperature-dependent interconversion of the two sugars.

For the determination of sorbitol concentration, neutralised $HClO_4$ extracts were made as described by Lowry and Passonneau [21]. Following centrifugation, sorbitol was measured by fluorimetric assay using sorbitol dehydrogenase (sheep liver: Sigma) according to the method of Clements et al. [22], in an Aminco-Bowman Spectrophotofluorimeter (American Instruments, Silver Spring, Maryland, USA).

Blood for determination of serum glucose and myo-inositol concentrations was collected from the tail vein. Serum glucose was determined using a glucose test kit (Boehringer, London, UK). Serum myo-inositol concentration was measured in $ZnSO_4$-$Ba(OH)_2$ precipitates as described by Clements and Stockard [17]. Aliquots of the supernatant following centrifugation were lyophylised and subjected to gas chromatography as described previously [14].

For determination of the myo-inositol content of the rat diet, triplicate 500 mg samples of finely powdered diet were extracted three times for 30 min each time into 10 ml of distilled water in a boiling water bath. Samples were centrifuged between extractions and the supernatants were pooled. The extracts were lyophylised, reconstituted in 5 ml of distilled water and deproteinised by the addition of $ZnSO_4$ (0.19 mol/l, 1 ml) and $Ba(OH)_2$ (0.3 mol/l, 1 ml). Aliquots of the supernatant following centrifugation were lyophylised with 50 μg of methyl-α-mannopyranoside as internal standard and subjected to gas chromatography as described previously [14].

Statistical Analysis

Results are expressed as mean ± SEM. The significance of difference between the means was calculated using an unpaired Student's t-test with 95% confidence limits.

Results

Animals

Control rats in Trial 1 gained weight throughout the course of the experiment (Table 1). However, the diabet-

Table 1. Animal weight, serum glucose and myo-inositol concentrations of control and streptozotocin-diabetic rats and the effect of sorbinil or myo-inositol treatment for 10 weeks

Animal group	Weight (g)		Serum glucose (mmol/l)	Serum myo-inositol (μmol/l)
	Start	End		
Trial 1				
Control rats	312 ± 3 (13)	365 ± 7 (13)	9.8 ± 0.5 (12)	48.6 ± 4.9 (12)
Diabetic rats	318 ± 3 (10)	220 ± 10 (10)[a]	46.9 ± 4.0 (6)[a]	71.9 ± 16.8 (6)
Diabetic/sorbinil rats	316 ± 3 (12)	237 ± 10 (12)[a]	48.4 ± 2.4 (11)[a]	60.2 ± 3.7 (10)
Trial 2				
Control rats	284 ± 5 (20)	358 ± 6 (20)	9.1 ± 0.3 (20)	74.0 ± 2.6 (16)
Diabetic rats	285 ± 6 (12)	283 ± 7 (12)[a]	28.9 ± 1.1 (11)[a]	78.9 ± 8.7 (11)
Diabetic/sorbinil rats	278 ± 5 (12)	282 ± 8 (10)[a]	29.5 ± 1.0 (9)[a]	111 ± 9.6 (8)[a,c]
Diabetic/inositol rats	288 ± 3 (10)	290 ± 6 (7)[a]	28.2 ± 1.1 (7)[a]	197 ± 42.0 (7)[a,b]

Results are expressed as mean ± SEM with number of animals in parentheses.
[a] $p < 0.001$ versus control; [b] $p < 0.02$ versus diabetic; [c] $p < 0.01$ versus diabetic

Table 2. Sorbitol, fructose, glucose and myo-inositol concentrations in rat sciatic nerve during streptozotocin diabetes and the effect of sorbinil treatment

Animal group	Fructose (μmol/g wet weight)	Sorbitol (μmol/g wet weight)	Glucose (μmol/g wet weight)	Myo-Inositol (μmol/g wet weight)
Trial 1: 2 weeks following induction of diabetes				
Control rats	–	0.13 ± 0.01 (8)	1.91 ± 0.13 (10)	3.40 ± 0.18 (10)
Diabetic rats	4.46 ± 0.31 (9)	1.47 ± 0.20 (9)[b]	8.24 ± 0.56 (9)[b]	2.51 ± 0.10 (9)[a]
Diabetic/sorbinil rats	1.72 ± 0.31 (5)[d]	0.07 ± 0.01 (6)[b, d]	10.60 ± 0.39 (9)[b, c]	3.64 ± 0.16 (9)[c]
Trial 1: 10 weeks following induction of diabetes				
Control rats	1.81 ± 0.13 (6)	0.25 ± 0.01 (13)	2.92 ± 0.07 (6)	3.63 ± 0.15 (6)
Diabetic rats	7.49 ± 0.42 (9)[b]	2.38 ± 0.17 (9)[b]	9.36 ± 0.62 (9)[b]	2.40 ± 0.18 (9)[b]
Diabetic/sorbinil rats	3.17 ± 0.23 (10)[b,d]	0.51 ± 0.09 (11)[a,d]	10.85 ± 0.35 (10)[b]	3.56 ± 0.10 (10)[d]

Results are expressed as mean ± SEM with number of animals in parentheses.
[a] $p < 0.01$ versus control; [b] $p < 0.001$ versus control; [c] $p < 0.01$ versus diabetic; [d] $p < 0.001$ versus diabetic

ic and sorbinil-treated groups lost weight to 69% and 75% of their initial weights, respectively. Serum glucose was markedly and significantly elevated in both the diabetic and sorbinil-treated diabetic groups at the end of the trial. The glucose concentration was higher than anticipated with this dose of streptozotocin and as a result the animals were in extremely poor condition with some deaths during the course of the trial. Serum myo-inositol concentration was elevated in the diabetic and sorbinil-treated groups, although significantly so only in the former.

Due to the severity of diabetes of the animals in Trial 1, the streptozotocin dose was reduced for Trial 2. The elevation of serum glucose at the end of the trial in the diabetic, sorbinil-treated diabetic and myo-inositol-treated diabetic groups was less than that of Trial 1. The diabetic animals in this trial showed no change in body weight by the end of the experiment, while the control group gained weight. At the end of the trial, serum myo-inositol concentration was not significantly different between control and diabetic groups, but was significantly elevated compared with controls in both the sorbinil-treated and myo-inositol-treated diabetic groups.

Trial 1: Sciatic Nerve Sugar and Polyol Concentration and Motor Nerve Conduction Velocity

Sugar and polyol concentrations were measured in sciatic nerves 2 weeks after the start of the trial to confirm changes in these compounds in diabetic rat nerve and to assess the potency of sorbinil treatment. Consistent with previously reported results [2, 14], sorbitol, fructose and glucose concentrations were elevated and myo-inositol concentration decreased in the nerves of the diabetic group after 2 weeks (Table 2). Treatment of diabetic animals with sorbinil for 2 weeks completely prevented the rise in sorbitol concentration and reduced fructose levels, although not to normal. Sorbinil treatment resulted in a significant increase in nerve glucose concentration compared with the diabetic group ($p < 0.05$) and elevated myo-inositol concentration to a level similar to that of control rats.

The rise in nerve sorbitol concentrations after 10 weeks of diabetes was similar to that after 2 weeks (approximately tenfold) although absolute concentrations were higher. However, sorbinil at the dose used in this study and at the extremely high serum glucose con-

Table 3. Serial motor nerve conduction velocity (MNCV) in rats during streptozotocin diabetes and the effect of sorbinil treatment

	MNCV (m/s)			
	Week			
	0	2	4	10
Control rats ($n=13$)	38.5 ± 1.08	40.1 ± 0.92	39.9 ± 1.06	42.6 ± 0.52
Diabetic rats ($n=9$)	40.4 ± 1.10	39.2 ± 1.07	38.2 ± 1.09	34.5 ± 0.71[b]
Diabetic/ sorbinil rats ($n=10$)	40.0 ± 1.18	38.8 ± 0.61	36.9 ± 0.96[a]	38.7 ± 0.73[b,c]

Results are expressed as mean ± SEM. [a] $p<0.05$ versus control; [b] $p<0.001$ versus control; [c] $p<0.001$ versus diabetic rats

Table 4. The effect of short-term sorbinil or myo-inositol treatment on nerve myo-inositol concentration of streptozotocin-diabetic rats

Animal group	Myo-Inositol (μmol/g wet weight)
Control rats	3.52 ± 0.04 (5)
Diabetic rats	3.03 ± 0.08 (4)[b]
2-day sorbinil/diabetic rats	3.47 ± 0.23 (3)
7-day sorbinil/diabetic rats	3.47 ± 0.25 (3)
2-day inositol/diabetic rats	3.37 ± 0.01 (3)[a]
7-day inositol/diabetic rats	3.78 ± 0.06 (3)[a]

Results are expressed as mean ± SEM with number of animals in parentheses. Diabetes was of 6 months duration and was induced by a single IP injection of streptozotocin (35 mg/kg body weight). [a] $p<0.02$ versus control; [b] $p<0.001$ versus control

centrations of these animals did not completely inhibit the enzyme, the reduction in sorbitol concentration being 88%. Fructose concentration was significantly higher than in control rats. Consistent with results obtained at 2 weeks, sorbinil treatment prevented a fall in myo-inositol concentration which had decreased by approximately 30% in the untreated diabetic rats after 10 weeks of diabetes.

During the 10-week trial, MNCV of the control group increased significantly ($p<0.01$; Table 3). In contrast, MNCV of the untreated diabetic group decreased gradually throughout the trial, although a statistically significant decrease was attained only by week 10. Sorbinil treatment of diabetic rats did not prevent a decrease in MNCV and the difference compared with controls was significant at week 10. However, there was an improvement in MNCV of the sorbinil-treated group between weeks 4 and 10 (NS) and MNCV at week 10 was significantly higher than that of the untreated diabetic animals.

Trial 2: Sciatic Nerve Sugar and Polyol Concentrations and Motor Nerve Conduction Velocity

In view of the finding that sorbinil treatment of diabetic rats restores sciatic nerve myo-inositol concentration to normal (Table 1), Trial 2 included a myo-inositol-treated diabetic group as a comparison to sorbinil treatment. Detailed nerve sugar and polyol results were presented as Table 2 in the preliminary report [20] and are not repeated here.

Reduction of the streptozotocin dose resulted in lower serum glucose concentrations in the diabetic rats (Table 1). Sciatic nerve glucose concentration was reduced compared with that of Trial 1 (10 week measurement), although it was significantly higher than that of control rats. Nerve fructose and sorbitol concentrations in the untreated diabetic group were significantly elevated, the extent of the increase of the latter being unaccountably more than that seen in Trial 1 where ambient glucose concentrations were higher (Tables 1 and 2). However, the increased sorbinil dose administered to the diabetics was sufficient to restore both increased sorbitol and decreased myo-inositol concentrations to control values. Indeed restoration of normal sorbitol concentrations in 2 week diabetic rats (75 mg/kg streptozotocin) was achieved after only 3 days of sorbinil treatment (20 mg · kg^{-1} · day^{-1}) (control rats: not measurable (by gas chromatography), diabetic rats: 1.15 μmol/g, sorbinil-treated diabetic rats: not measurable) and 2 days of this treatment of 6 month diabetic rats (35 mg/kg streptozotocin) returned myo-inositol concentration to levels not significantly different from control values (Table 4).

Myo-Inositol treatment raised serum myo-inositol concentrations almost threefold (Table 1); this resulted in a normalisation of sciatic nerve myo-inositol concentration which was achieved in 6-month diabetic rats after 7 days of treatment (Table 5). This treatment regimen had no effect on sorbitol, fructose or glucose concentrations in sciatic nerve.

Initial MNCVs of the control rats and those which subsequently became diabetic were similar (Table 5). These results were re-calculated from the experiments of Table 1 of the preliminary account [20], omitting values from rats which died later in the trial. Six weeks following the induction of diabetes, MNCV of both groups had increased, although that of the diabetic group was elevated to a significantly smaller extent (control versus total diabetic group, $p<0.001$). At this point the diabetic group was divided into three subgroups with similar MNCV's which were significantly different from controls as described in the Methods section. MNCV of the control and diabetic groups did not alter significantly during the 9-week treatment period. At all times during the treatment period MNCV of the untreated diabetics was significantly slowed compared with that of control rats. Sorbinil treatment of diabetics for 3 weeks did not alter MNCV (Table 5). However, continuing treatment with sorbinil induced a gradual improvement in MNCV which was not significantly different from controls at the end of the treatment period. In contrast with sorbinil treatment, administration of myo-inositol for 3 weeks returned MNCV to normal

Table 5. Serial motor nerve conduction velocity (MNCV) in rats during streptozotocin diabetes and the effect of myo-inositol or sorbinil treatment in Trial 2: treatment with sorbinil or inositol began at Week 6

Animal group	Pre-treatment (weeks)		Post-treatment (weeks)		
	0	6	3	6	9
Control rats ($n=17$)	36.9 ± 0.72	43.1 ± 0.68	41.7 ± 0.48	42.7 ± 0.57	43.0 ± 0.52
Diabetic rats	36.6 ± 0.64 (28)	39.5 ± 0.66 (12)[c]	36.7 ± 0.92 (12)[c]	38.9 ± 0.49 (12)[c]	39.0 ± 0.74 (12)[c]
Sorbinil/diabetic rats ($n=9$)		39.6 ± 0.95[b]	39.6 ± 1.12	42.2 ± 0.92[d]	43.3 ± 0.50[e]
Inositol/diabetic rats ($n=7$)		38.8 ± 1.44[a]	41.1 ± 0.99[d]	42.3 ± 0.89[d]	42.9 ± 0.55[e]

Results are expressed as mean ± SEM with number of animals in parentheses.
[a] $p<0.02$ versus control; [b] $p<0.01$ versus control; [c] $p<0.001$ versus control; [d] $p<0.01$ versus diabetic; [e] $p<0.001$ versus diabetic

and this improvement was maintained until the end of treatment (Table 5). MNCV of the sorbinil-treated diabetic group was significantly higher than that of the untreated diabetic group after 6 and 9 weeks of treatment while MNCV of the myo-inositol-treated diabetic group was higher throughout the treatment period.

Discussion

Sorbinil proved to be a potent inhibitor of aldose reductase in vivo since it completely prevented a rise in sciatic nerve sorbitol concentration during the early stages of experimental diabetes. This prevention was not maintained during the 10-week experimental period, but this may have been due to an insufficiently high dose of sorbinil or to the extremely high serum glucose concentrations in these animals. However, despite persistent hyperglycaemia and elevated nerve glucose concentrations, sorbinil treatment resulted in an 88% inhibition of the increase in sorbitol concentration and a reduction in fructose levels. Not only could sorbinil treatment prevent or reduce the extent of elevation in nerve sorbitol concentrations, it was found also to reduce efficiently already elevated sorbitol concentrations. This reduction appeared to be complete only 3 days after initiation of treatment. Reversal of increased sorbitol concentrations also resulted in a near-normalisation of nerve fructose levels.

Sorbinil treatment consistently increased nerve glucose concentration although this was significantly different on only one occasion compared with untreated diabetic rats. This increase may simply be caused by the inhibition of glucose entry into the polyol pathway. It is not known if this exacerbation of already high glucose concentrations results in further biochemical alterations.

Prevention of the elevation in sorbitol concentrations during experimental diabetes also prevented loss of free myo-inositol from peripheral nerve and this effect was maintained until the end of the trial despite a small significant rise in sorbitol concentrations. Reversing already elevated sorbitol concentrations with sorbinil treatment also resulted in a near normalisation of myo-inositol concentration which was reduced by 30% in untreated animals at the end of the trial. Treatment of diabetic rats with sorbinil for only 2 days was sufficient to increase nerve myo-inositol concentration to levels similar to controls, which suggests that the accumulation of sugar alcohols in peripheral nerve during experimental diabetes may be responsible for the loss of myo-inositol. Experiments in vitro suggest that this may be through an effect on the active transport of inositol [23], a process which has been described independently by Greene and Lattimer [24].

Modification of the transport mechanism for myo-inositol in peripheral nerve in vivo is evident since elevation of serum myo-inositol concentration by dietary myo-inositol results in near-normalisation of nerve myo-inositol levels. The latter has been demonstrated previously using inositol-supplemented diets [2, 25, 26]. The control serum inositol concentration of Trial 1 was unexpectedly low in comparison with our earlier finding of 80 μmol/l [14]. Trial 2 gave a control figure of 74 μmol/l. These concentrations are higher than those of Greene et al. [2], possibly because our rat diet had 0.022% inositol by weight, while theirs had only 0.011% inositol. In our hands, tail-vein and cardiac blood samples gave similar concentrations of inositol. In the present study, elevation of nerve myo-inositol concentration in myo-inositol-treated diabetic rats was observed after 2 days of treatment and 7 days of treatment results in complete normalisation. In both trials described here, serum myo-inositol also was elevated during sorbinil treatment. The possibility therefore exists that sorbinil treatment increased nerve myo-inositol concentration and conduction velocity through increased serum myo-inositol concentration with resultant increased transport. However, our study in vitro [23] suggests that sorbinil partly reverses glucose-inhibited myo-inositol transport with no elevation in medium myo-inositol concentration.

Although peripheral nerve sorbitol and myo-inositol were maintained near-normal throughout a 10-week period, sorbinil treatment was unable to prevent gradual slowing of MNCV in diabetic rats although a slight improvement was evident late in the trial. It was not possible to investigate this further, since the animals were in extremely poor condition and the trial was therefore terminated. However, it was found that both

sorbinil and myo-inositol treatment could reverse slowed MNCV in diabetic rats over 9 weeks of treatment. Since this reversal was possible in the myo-inositol-treated group without reduction of nerve sorbitol and since myo-inositol was normalised in both cases, it appears that elevated nerve sorbitol is unconnected with the slowing in MNCV, but may lead to this through its effect on myo-inositol metabolism.

A return to normal of nerve sorbitol concentration and MNCV in diabetic rats following sorbinil treatment [27] or prevention of slowing of MNCV with ICI 105552 treatment [28] have been reported recently and this study confirms these former results. However, some controversy exists concerning the effect of myo-inositol [2, 5, 25, 26]. We have achieved normal values of MNCV by different treatments which both restore nerve myo-inositol concentrations; this further strenghthens the argument for the involvement of myo-inositol and the polyphosphoinositides in peripheral nerve function.

Extrapolation of these results to treatment of human diabetic neuropathy should be made with caution. Results with either of the two treatments have not proved entirely encouraging. Clinical trials with myo-inositol treatment have shown results ranging from no effect [29], to improvement in some electrophysiological parameters [30, 31]. Human trials using various aldose reductase inhibitors also have proved inconsistent, with effects varying from no change in conduction velocities to improvement in both sensory and motor conduction [32–35]. However, analysis of post-mortem material [36, 37] and human nerve biopsies [38] suggests that alterations of polyol pathway activity and myo-inositol metabolism occur in nerves from human diabetics and encourages further experiments with these treatments.

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