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Prevention of Defects of Axonal Transport and Nerve Conduction Velocity by Oral Administration of *myo*-Inositol or an Aldose Reductase Inhibitor in Streptozotocin-Diabetic Rats

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Summary. The effects of orally-administered *myo*-inositol have been compared with those of an aldose reductase inhibitor on acute neurological defects in experimentally diabetic rats. Three groups of streptozotocin-treated diabetic rats (50 mg/kg, IP) together with three groups of age-matched controls (saline, IP) were compared. One pair of groups (control and diabetic) were untreated for 3 weeks, another pair of groups received daily oral *myo*-inositol (667 mg/kg) and the third pair received an aldose reductase inhibitor (ICI 105552; 50 mg·kg^{-1}·day^{-1}, orally). The untreated diabetic group showed statistically significant deficits in accumulation, proximal to 24 h sciatic nerve constrictions, of choline acetyltransferase activity by comparison with untreated controls (2.8 ± 0.4 versus 5.1 ± 0.4 nmol acetylcholine·h^{-1}·nerve^{-1}; $p < 0.001$). The untreated diabetic rats also showed a fall in motor nerve conduction velocity of 6.2 ± 0.7 m/s which was statistically significant ($p < 0.001$). Treatment of the diabetic group with *myo*-inositol prevented the development of both defects of axonal transport and conduction velocity; both measurements were similar to those of the *myo*-inositol treated control rats. Likewise the diabetic rats which received aldose reductase inhibitor showed prevention of both defects. Nerves from untreated diabetic rats showed marked sorbitol accumulation and a statistically significant reduction in *myo*-inositol content by comparison with the untreated controls (sorbitol, 1.56 ± 0.22 versus 0.8 ± 0.01 and *myo*-inositol, 1.47 ± 0.10 versus 2.3 ± 0.10 nmol/mg; $p < 0.001$). Treatment of the diabetic rats with *myo*-inositol elevated the *myo*-inositol levels in nerve (3.05 ± 0.22 nmol/mg) without affecting nerve sorbitol levels. Treatment of diabetic rats with aldose reductase inhibitor also normalised nerve *myo*-inositol (2.75 ± 0.20 nmol/mg) and reduced nerve sorbitol (0.08 ± 0.01 nmol/mg). These findings are consistent with the suggestion that both treatments prevent the development of the two functional defects by normalising the nerve *myo*-inositol content.

Key words: Diabetic neuropathy, axonal transport, nerve conduction, nerve crush, *myo*-inositol, aldose reductase.

Investigation into the causes of diabetic neuropathy has stimulated interest in three areas of study. Firstly, there have been a number of demonstrations of disordered axonal transport in the nerves of experimentally diabetic rats [17]. Secondly, several groups of workers have shown that inhibitors of the enzyme aldose reductase prevent or ameliorate some of the acute neurological defects seen in diabetic animals [20, 25] and patients [4, 11]. Thirdly, there has been the discovery that administration of *myo*-inositol to diabetic rats reverses the reduction in nerve *myo*-inositol content associated with diabetes and attenuates or abolishes nerve conduction velocity defects [8, 9]. We have sought to examine possible links between these phenomena. In a preliminary study we found that administration of an inhibitor of aldose reductase, over the first 3 weeks of streptozotocin-induced diabetes in rats, prevented the development not only of nerve conduction defects but also of reduced orthograde axonal transport [20]. The present study was designed to examine the effects of normalisation of nerve *myo*-inositol content, by oral administration of the substance, on axonal transport and nerve conduction in acute experimental diabetes in rats. The second objective was to compare the effects of *myo*-inositol treatment with those of an aldose reductase inhibitor by investigating the effect of the latter on nerve *myo*-inositol content.

Materials and Methods

Male Wistar rats (290–310 g) were fasted overnight and made diabetic by a single injection of streptozotocin (50 mg/kg, IP) the following morning. The drug was dissolved in 0.9% saline immediately before injection. Age- and weight-matched control groups were treated iden-

tically except that saline was injected. Three days later blood samples were taken from the streptozotocin-treated animals fasted for 2 h to obviate post-prandial peaks of glucose. Blood samples and glucose standards (50 µl) were collected into ice-cold 0.016% uranyl acetate (1 ml). The mixture was agitated, centrifuged at 800 *g* and the supernatant assayed for glucose spectrophotometrically by the standard glucose oxidase method (assay kit: 'GOD-PAP', Boehringer Ingelheim, London). Animals with blood glucose <15 mmol/l were excluded from further study.

Experimental Organisation and Treatment of Animals

In view of previous findings [20] experiments were designed to examine the effects of 3 weeks of diabetes. Since it is possible to measure motor nerve conduction velocity (MNCV) in a recovery experiment (see below), two measurements of MNCV per rat were made, one 24 h before injection of streptozotocin, the other 21 days later. Earlier work [20] had shown that, in untreated diabetic rats, MNCV falls to a plateau value over this time course. Two measurements of MNCV were likewise made 21 days apart in non-diabetic control rats.

Treatment with the aldose reductase inhibitor or with *myo*-inositol was begun on the day of injection of streptozotocin or saline. Thus one group of diabetic rats and its control group were given a single daily dose (50 mg/kg, orally) of an aldose reductase inhibitor (ICI 105552; 1-(3,4-dichlorobenzyl)-3-methyl-1, 2-dihydro-2-oxquinol-4-yl-acetic acid). A second diabetic group and its control were given daily 667 mg/kg *myo*-inositol orally (Sigma (London) Chemicals, Poole, Dorset, UK). Both compounds were made up in distilled water daily. These two treatments were maintained for 21 days; a third pair of diabetic and control groups were left untreated over a similar period of time.

Twenty-one days after injection of streptozotocin (or saline) the left sciatic nerve of each rat was constricted for the measurement of axonal transport. The animals were sacrificed 24 h later and blood samples were taken for glucose assay.

Measurement of Motor Nerve Conduction Velocity

The procedure is described in full elsewhere [16]. Rats were anaesthetized with halothane using a Boyle's Apparatus (British Oxygen, London, UK) driven with O_2 and set to 5% halothane for induction and 1%–2% for maintenance of anaesthesia. The left sciatic nerve was stimulated first at the sciatic notch and then at the Achilles tendon. Stimulation comprised single 0.1 ms pulses of amplitude 1–4 V delivered via fine percutaneous needle electrodes. Consequent to each stimulation an electromyogram (EMG) was recorded, again using fine needle electrodes, from the second interosseus muscle via a 250 × gain AC preamplifier (Scientific and Research Instruments, Croydon, Surrey, UK) on a single beam storage oscilloscope (Model 5103N, Tektronix, Beaverton, Oregon, USA). The temporal separation of the peaks of the EMGs, induced by stimulation at the two sites, was measured using dividers. The mean of six measurements was taken on each occasion. Nerve length from sciatic notch to Achilles tendon was measured at death and the distance was used to calculate MNCV in m/s.

Rectal temperature was monitored throughout anaesthesia for this procedure (2–4 min). No hypothermia was observed, all rats remaining between 37 and 38 °C.

Constriction of the Sciatic Nerve

The left sciatic nerve was ligated for 24 h to measure the accumulation of choline acetyltransferase activity proximal to the constriction as an index of orthograde axonal transport of the enzyme. Under halothane anaesthesia and using aseptic techniques the left sciatic nerve was exposed via a 1-cm incision in the flank. A prolene suture (0.7 metric: Ethicon, Livingston, W. Lothian, Scotland) was passed around the nerve and tied tightly, level with the mid-point of the femur. The wound was closed with Michel clips (Thackray, Leeds, UK) and the animal allowed to recover. This procedure took no longer than 5 min from the onset of anaesthetic induction.

The rats were killed 24 h later by a blow on the head and bled from the throat. Both sciatic nerves were removed rapidly and placed on an ice-cold stainless steel plate. The constricted nerve was cut into 3 mm segments to give three segments proximal and two segments distal to the ligature. From the contralateral nerve two 3 mm segments were cut for comparison with the two segments immediately proximal to the ligature in the constricted nerve. These seven segments of nerve were homogenized for enzyme assay as described below. The remaining portions of the unconstricted nerve were processed for measurement of sorbitol and *myo*-inositol.

Assay of Choline Acetyltransferase

The method used was that described by Fonnum [5] with minor modification. Each nerve segment was homogenized in ice-cold buffer [5] in a glass-in-glass hand homogenizer. The homogenates were centrifuged at 1400 *g* for 10 min at 4 °C. Aliquots (50 µl) of each supernatant were reacted in duplicate with 50 µl of the 'working buffer' [5] containing (per 1 ml) 0.2 µmol acetyl-coenzyme A (incorporating 0.5 µCi ^{3}H-acetyl-coenzyme A), 56 nmol choline iodide and 0.3 µmol eserine sulphate. The reaction was allowed to proceed for 30 min at 25 °C and was arrested by transfer of the reaction mixture to ice-cold phosphate buffer (5.0 ml, 0.1 mol/l; pH 7.4). Radiolabelled acetylcholine was separated from unconverted acetyl-coenzyme A and measured by liquid scintillation counting [5]. Enzyme activity was calculated in units of nmol acetylcholine produced per hour per sample.

Acetyl-coenzyme A (trilithium salt) was obtained from Boehringer, Lewes, East Sussex, UK; *acetyl*-^{3}H acetyl-coenzyme A from New England Nuclear, Southampton, UK; all other chemicals were purchased from Sigma, Poole, Dorset, UK.

Calculation of Accumulations

Accumulation of enzyme activity was restricted to the two nerve segments (total length 6 mm) proximal to the ligature. These segments are coded P_1 and P_2. From the sum of activities in P_1 and P_2 was subtracted the sum of the activities in the two segments taken, at the equivalent level, from the unconstricted contralateral nerve. Thus a net accumulation for each rat was obtained.

Measurement of Sorbitol and myo-Inositol Content

The bulk of the non-constricted right sciatic nerve was placed on a stainless steel plate at 4 °C, dissected free of fat and connective tissue, blotted and weighed quickly on a pre-tared micro-balance. The nerve was then boiled for 20 min in distilled water (1 ml) containing α-methyl mannoside (30 µg/ml; Sigma) as an internal standard. A mixture of external standards; α-methyl mannoside, sorbitol, *myo*-inositol (all 30 µg/ml; Sigma) – was similarly treated in parallel. The mixtures were cooled and deproteinized by addition of ZnS04 (0.2 ml, 0.19 mol/l) followed 10 min later by $Ba(OH)_2$ (0.2 ml, 0.2 mol/l). The samples were centrifuged at 800 *g* for 10 min and the supernatants were freeze-dried. The lyophilisates were silylated under a mixture of pyridine, hexamethyldisilazane and trimethylchlorosilane (10:2:1, v/v; 0.5 ml; Pierce&Warriner, Chester, UK) [19]. After 24 h incubation at room temperature, distilled water (2 ml) and cyclohexane (0.2 ml, spectroscopic grade: British Drug Houses Chemicals, Poole, Dorset, UK) were added and the samples vortex mixed. After centrifugation at 800 *g* for 2 min, the cyclohexane phase was aspirated off for chromatography.

Extracts were assayed in duplicate using 3% OV3 on Chromosorb W (HP), 80/100 mesh (Pierce&Warriner, Chester, UK) in a gas chromatograph fitted with a flame ionisation detector (Model F17, Perkin-Elmer, Beaconsfield, Buckinghamshire, UK). Thermal programming was used to enhance the peak height for *myo*-inositol. The column was held at 180 °C for 2 min after sample injection and the temperature was then raised at a rate of 4 °C/min for 5 min. *Myo*-inositol was eluted finally at 200 °C. Recovery of sorbital and *myo*-inositol was calculated from that of the internal standard, α-methyl mannoside and was 80%–96%. No marked within-run or day-to-day variation in recovery or sensitivity was seen.

Statistical Analyses

All results are presented as mean ± SEM. Examination of the significance of changes in MNCV was made using paired t-tests. Comparisons of group means for other variables were made using unpaired t-tests. Linear regression analyses were performed by the method of least squares.

Results

Body Weight and Blood Glucose

The changes in body weight over the experimental period of 21 days together with the final blood glucose concentrations are shown for all groups of rats in Table 1. These data show that the streptozotocin-treated rats exhibited the classical weight loss and hyperglycaemia of this form of diabetes and that treatment of diabetic rats with either aldose reductase inhibitor or with *myo*-inositol had no effect on the weight loss nor upon the severity of the hyperglycaemia. Three rats died within 3 days of injection of streptozotocin. No further deaths or apparent morbidity were observed.

Motor Nerve Conduction Velocity (MNCV)

The mean MNCV for all rats at the point of entry into the study was 49.8 ± 0.6 m/s ($n = 54$). The changes in MNCV in each group are given in Table 1. These data show that there was no change over the experimental period in the MNCV of untreated control rats whilst the untreated diabetic group showed a fall of 6.2 ± 0.7 m/s which was highly significant ($p < 0.001$). The two treated control groups showed small, statistically insignificant increases in MNCV over the 21 days. Treatment of diabetic rats with aldose reductase inhibitor or with *myo*-inositol resulted in there being no significant change in MNCV over the 21 days of diabetes. Thus these treatments prevented the fall in MNCV characteristic of untreated diabetes.

Axonal Transport of Choline Acetyltransferase

The choline acetyltransferase activities measured in each nerve segment are shown in Figure 1 and the means of the calculated accumulations of activity are given in Table 1. The latter indicates that untreated diabetes caused a statistically significant reduction in choline acetyltransferase accumulation by comparison with the value obtained from the untreated control group ($p < 0.001$ by unpaired t-test). The segmental distribution of choline acetyltransferase activity in the nerves of these two groups of rats (Fig. 1) shows that this reduced accumulation arose principally from a lower activity than controls in segment P_1 ($p < 0.02$). The activity measured in the segments of unconstricted nerve was higher in untreated diabetics than in their control group. This small difference contributed to the accumulation deficit, but was not in itself significant.

Treatment with the aldose reductase inhibitor had no effect on the accumulation or distribution of choline acetyltransferase activity in the nerves of control rats. However, the aldose reductase inhibitor prevented the abberant choline acetyltransferase distribution seen in the nerves of the untreated diabetic rats. Thus the choline acetyltransferase accumulation in the nerves of the treated diabetic rats was similar to that in untreated control and significantly greater than that in untreated diabetic rats (Table 1; $p < 0.01$). This accumulation was a manifestation of both an increase in P_1 activity and a decrease in unconstricted nerve activity.

The effects of oral administration of *myo*-inositol were even more marked. Treatment of the control rats produced a choline acetyltransferase accumulation which was numerically greater than that measured in the untreated controls, though the difference was not statistically significant. However, the activity in segment P_1 of the constricted nerves of the *myo*-inositol treated control rats was significantly greater than the P_1 segment activity of the untreated control group ($p < 0.001$). The lack of significant difference between the accu-

Table 1. Changes in body weight and motor nerve conduction velocity over 21 days with final blood glucose concentrations, accumulation of choline acetyltransferase activity and nerve sorbitol and *myo*-inositol contents for all groups of rats

	Change in body weight (g)	Blood glucose concentration on day 21 (mmol/l)	Change in MNCV (m/s)	Accumulation of choline acetyltransferase activity (nmol acetylcholine h^{-1}nerve^{-1})	Sorbitol content (nmol/mg nerve)	*myo*-inositol content (nmol/mg nerve)
Untreated controls ($n = 10$)	+13.8 ± 1.8	6.3 ± 0.2	+2.2 ± 1.5	5.1 ± 0.4	0.08 ± 0.01	2.30 ± 0.10
Untreated diabetics ($n = 11$)	−39.5 ± 7.1	22.5 ± 1.4	−6.2 ± 0.7[a]	2.8 ± 0.4	1.56 ± 0.22	1.47 ± 0.10
Aldose reductase inhibitor-treated controls ($n = 10$)	+17.0 ± 2.3	4.7 ± 0.3	+2.1 ± 1.3	4.2 ± 0.4	< 0.05	2.51 ± 0.12
Aldose reductase inhibitor-treated diabetics ($n = 7$)	−24.0 ± 4.5	27.5 ± 1.2	+2.6 ± 1.7	4.7 ± 0.5	0.08 ± 0.01	2.75 ± 0.20
myo-Inositol-treated controls ($n = 9$)	+24.0 ± 2.9	5.0 ± 0.7	+3.4 ± 1.8	6.2 ± 0.7	0.10 ± 0.01	2.77 ± 0.24
myo-Inositol-treated diabetics ($n = 7$)	−37.8 ± 9.8	26.3 ± 2.0	+1.1 ± 2.0	6.7 ± 0.6	1.45 ± 0.13	3.05 ± 0.22

Body weight and motor nerve conduction velocity (MNCV) values are mean ± SEM of the differences between two measurements made the day before onset of treatment and 21 days later. [a] $p < 0.001$ (paired t). By the unpaired t test, the untreated diabetic rats were significantly lower in

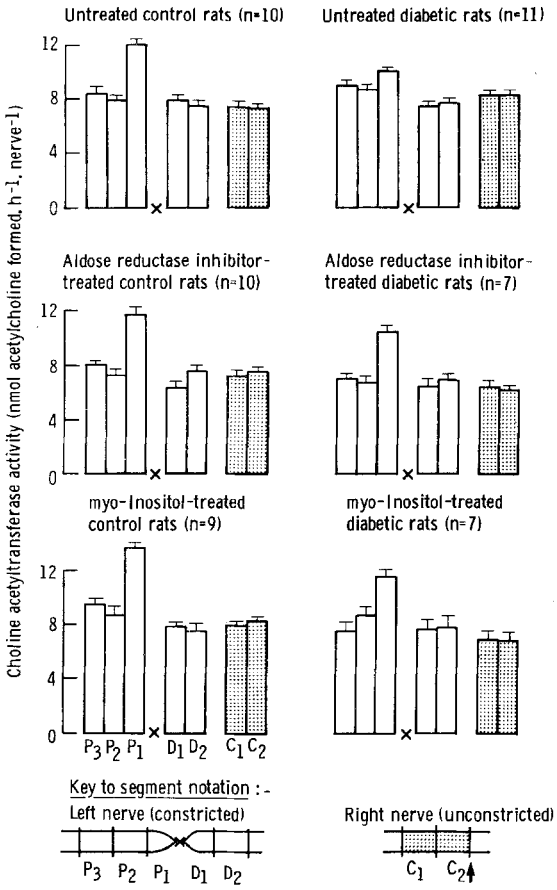

Fig. 1. The distribution of choline acetyltransferase activity between segments of constricted ( ) and contralateral unconstricted ( ) sciatic nerves of six groups of control and diabetic rats. Limit bars denote SEM. Nerve segments (3 mm in length) are coded with respect to the constriction (X) as indicated in the lower left-hand histogram and the key diagram. Arrow indicates the point on the unconstricted nerve level with the constriction on the contralateral trunk

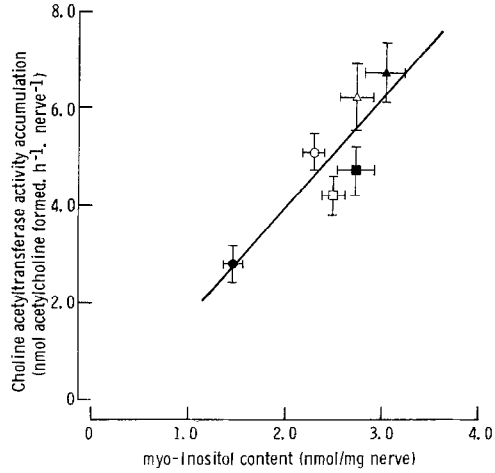

Fig. 2. Regression of choline acetyltransferase activity, accumulated proximal to constrictions in ligated left sciatic nerves, against *myo*-inositol content of the contralateral sciatic nerve. The regression line was calculated from pairs of coordinates for individual animals. Group mean values are shown: filled symbols, diabetic, open symbols, control rats; circles, untreated ($n=10$ controls, $n=11$ diabetics); triangles, *myo*-inositol treated ($n=9$ controls, $n=7$ diabetics) and squares, rats treated with aldose reductase inhibitors ($n=10$ controls, $n=7$ diabetics). Limit bars denote SEM

mulations of activity in these two groups is attributable to the slight elevation of activity in the unconstricted nerves in the *myo*-inositol treated group. Treatment of diabetic rats with *myo*-inositol markedly elevated the accumulation of choline acetyltransferase activity ($p<0.001$). This treatment did not produce a rise in the P_1 segment activity by comparison with untreated diabetic rats, but the increase occurred together with a reduction in the activity present in unconstricted nerve. Again the latter did not attain statistical significance, but the combination of the two additive effects gave rise to a significant difference in accumulation.

Nerve Content of Sorbitol and myo-Inositol

The content of sorbitol and *myo*-inositol in the unconstricted sciatic nerves of all rats are given in Table 1. Comparison of untreated control with untreated diabetic rats shows that the latter accumulated a large amount of sorbitol in their nerves and this was accompanied by a deficit of *myo*-inositol. Both changes were highly significant ($p<0.001$). Treatment of control rats with the aldose reductase inhibitor reduced slightly the already very low nerve sorbitol content to a level which was just below the lower limit of the assay (0.05 nmol/mg nerve). Aldose reductase inhibitor treatment did not affect the *myo*-inositol content of the nerves from the control rats, whereas in the diabetic group it completely prevented the accumulation of sorbitol and surprisingly normalized the nerve content of *myo*-inositol. The levels of this sugar were significantly higher than those in the untreated diabetic group ($p<0.001$) and no different from those measured in the control rats which received the aldose reductase inhibitor.

Treatment of diabetic rats with *myo*-inositol prevented the reduction of nerve *myo*-inositol levels observed in the untreated diabetic group; the difference in nerve *myo*-inositol contents between treated and untreated diabetic rats being highly significant ($p<0.001$). Indeed, treatment of the diabetics with *myo*-inositol raised the nerve content of this sugar to significantly higher levels than those seen in untreated control rats ($p<0.01$). This treatment did not affect the accumulation of sorbitol, the levels being similar to those of untreated diabetic rats. Treatment of control rats with *myo*-inositol was without significant effect on either sorbitol or *myo*-inositol content of sciatic nerve.

Correlation of Variables

Linear regression analyses were performed to examine possible correlations between nerve *myo*-inositol content and accumulation of choline acetyltransferase activity proximal to the constriction and between nerve *myo*-inositol content and the change over 21 days in motor nerve conduction velocity. These analyses were performed on pairs of data from each animal. There was no significant correlation between nerve *myo*-inosi-

tol and conduction velocity; but the correlation between *myo*-inositol content and accumulation of choline acetyltransferase activity was highly significant (Fig. 2).

Discussion

This investigation has studied the effects of treatment of diabetic rats, either with an inhibitor of aldose reductase or with *myo*-inositol, on two functional neurological defects characteristic of acute diabetes, namely reduced motor nerve conduction velocity and impaired orthograde axonal transport. The existence of reduced motor nerve conduction velocity as an early complication of diabetes is well established both in man and in several species of animals with various forms of experimental diabetes. The particular axonal transport defect studied here has received much less attention but is also well established. Impaired orthograde axonal transport of choline acetyltransferase activity was first described some time ago [15] when a reduction of about 40% in accumulation proximal to a sciatic nerve constriction was seen after 3–4 weeks of streptozotocin-induced diabetes. In a previous study, we found a 39% reduction in choline acetyltransferase activity accumulation after 3 weeks of diabetes [20] which compares well with the reduction of 45% of the present investigation.

The wasting of the muscles of the flank in uncontrolled diabetes in rats reduces thermal insulation of the sciatic nerve. Selection of anaesthetic and control of ambient temperature are therefore important to preclude slowing of conduction due to nerve cooling. We have used local heating whilst monitoring nerve temperature via a needle thermistor to prevent cooling during barbiturate anaesthesia [20]. In pilot studies with halothane, normothermia was much more readily accomplished due to brisk induction and very short duration of anaesthesia. In the present study neither nerve nor rectal hypothermia were encountered. It is possible that nerve hypothermia could reduce axonal transport during the 24-h accumulation period when ambient temperature was not controlled. The only argument against this possibility is the finding that both the aldose reductase inhibitor and *myo*-inositol treatments gave normal axonal transport in diabetic rats with profound weight loss and muscle wasting.

The prevention of deficits of choline acetyltransferase accumulation and nerve conduction velocity by treatment of diabetic rats with ICI 105 552 is a confirmation of our earlier findings [20]. Inhibition of aldose reductase with Alrestatin has been found to ameliorate the motor nerve conduction velocity defect in galactose-fed rats [6] and to attenuate some nerve conduction defects in diabetic patients [4]. Sorbinil, another inhibitor of aldose reductase, which is structurally unrelated to ICI 105 552, also ameliorates the motor nerve conduction velocity deficit in diabetic rats [25] and diabetic patients [11]. It appears, therefore, that inhibition of aldose reductase has unequivocal beneficial effects on nerve conduction defects attributable to acute diabetes. In similar experiments, we have found that treatment of diabetic rats with Sorbinil not only prevents the development of deficient choline acetyltransferase activity accumulation, but also reverses the defect once it has been established at three weeks of untreated diabetes [12].

A deficiency of *myo*-inositol in the nerves of animals with acute experimental diabetes has been reported [3, 8, 13, 21, 23] and oral administration of *myo*-inositol has been shown to reverse the deficiency in nerve content and attenuate nerve conduction velocity defects [8, 9]. In the present context, this finding acquires added interest because of the effect of the aldose reductase inhibitor on nerve *myo*-inositol content. The two treated diabetic groups show the common feature that deficiency of nerve *myo*-inositol content was prevented by both treatments.

The implication of nerve *myo*-inositol content as an influence on axonal transport of choline acetyltransferase activity is supported by the highly significant correlation of the two variables (Fig. 2).

We consider that the beneficial effects of aldose reductase inhibition on acute neuronal dysfunction in diabetes may not be related directly to prevention of the accumulation of sorbitol or fructose. Instead, we suggest that the effects may be mediated, at least in substantial part, by correction of the nerve *myo*-inositol deficit. However, this does not preclude the possibility that hydrodynamic or biochemical consequences of sorbitol and fructose accumulation play some part in reducing the capacity of the nerve to accumulate and maintain a normal content of *myo*-inositol. It is otherwise difficult to understand how inhibitors of aldose reductase could act to normalize the nerve *myo*-inositol content. It is generally accepted that the reduced nerve content in diabetes arises as a result of competitive inhibition of uptake of *myo*-inositol by high levels of extracellular glucose. This phenomenon is widely reported for lens, kidney and gut [1, 2, 10, 21] and has more recently been shown to occur in peripheral nerve in vitro [7]. Thus, elevation of plasma *myo*-inositol, following oral administration of the substance, would be expected to reverse competitively the effects of hyperglycaemia on *myo*-inositol uptake by nerve. This hypothesis may be naive. Others have observed that some two weeks of treatment of diabetic rats with *myo*-inositol must elapse before a significant improvement in MNCV is seen [9]. Studies in our laboratory, involving treatment of diabetic rats with Sorbinil, in which we found that normalization of nerve *myo*-inositol content precedes by at least 1 week any improvement in either MNCV or axonal transport (unpublished work) support this view. This observation is not surprising, however, since the normalization of free *myo*-inositol in nerve is unlikely to have a direct effect on functional phenomena such as impulse conduction or axonal transport. The time lag may indicate secondary processes of biochemical repair such as incorporation of *myo*-inositol into phospholipids and return to normal of the activity of CDP-digly-

ceride: inositol transferase [3]. The influence of aldose reductase inhibition on the latter processes would be a profitable area of study.

The majoritiy of work concerned with nerve *myo*-inositol and aldose reductase inhibitors has focussed upon nerve conduction velocity as a functional manifestation of diabetes-induced neurological disorder. Thus interest has been restricted to acute neuropathies. The present implication of *myo*-inositol and aldose reductase in the development of defective axonal transport, may relate also to chronic neuropathy. The delivery of macromolecules to the periphery of the neurone by axonal transport is vital to the maintenance of the axon and its terminals [14]. Disorder of this process could contribute to degenerative neuronal dysfunction in diabetes, in which case, study of the effects of aldose reductase inhibitors and the processes whereby the nerve utilises *myo*-inositol and its phospholipid derivatives may be of benefit in understanding the aetiology and potential management of a variety of diabetic neuropathies. As yet there is little understanding of the factors which influence or regulate, under physiological conditions, conduction velocity and axonal transport. We have a similar limited view of the reasons underlying the importance of the high levels of *myo*-inositol in normal nerve. However, a recent report [18] suggests that a high level of *myo*-inositol may be necessary to prevent a restriction on phosphatidylinositol synthesis at sites of rapid turnover, where this process may be important for metabolic regulation of a variety of neurophysiological processes. The present findings vindicate the arguments of others [24] who predicted that aldose reductase inhibition might exert beneficial effects in nerve by normalizing *myo*-inositol content.

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