Treatment with an aldose reductase inhibitor can reduce the susceptibility of fast axonal transport following nerve compression in the streptozotocin-diabetic rat

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Summary. The effect of treatment with an aldose reductase inhibitor on the susceptibility of peripheral nerves to compression was studied in rats made diabetic by the injection of streptozotocin ($50 \text{ mg} \cdot \text{kg}^{-1}$). The response to nerve compression was determined in untreated diabetic rats after 22 days of diabetes and compared with the response in two similar groups of diabetic rats which had been treated with the aldose reductase inhibitor 'Statil' (ICI 128436; $25 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ orally) either from the induction of diabetes or for 7 days prior to nerve compression. Two groups of non-diabetic rats were treated with 'Statil' for either 22 days or 7 days to act as controls. Inhibition of fast axonally transported proteins was induced by local compression of the sciatic nerves 4 h after application of ^3H -leucine to the motor neurone cell bodies in the spinal cord. The inhibition of fast axonal transport was quantified by calculation of a transport block ratio.

Compression at 30 mmHg for 3 h induced a significantly greater ($p < 0.05$) inhibition of axonal transport at the site of compression in nerves of *untreated* diabetic rats (transport

block ratio 0.96 ± 0.24 , $n = 8$) than in nerves of control rats treated with the aldose reductase inhibitor for either the shorter time of 7 days (0.71 ± 0.17 , $n = 10$) or the longer time of 22 days (0.69 ± 0.08 , $n = 5$). In diabetic rats treated with the aldose reductase inhibitor for 22 days the inhibition (0.77 ± 0.12 , $n = 6$) was significantly less than that in *untreated* diabetic rats; treatment for 7 days reduced the transport block ratio to 0.85 ± 0.11 ($n = 8$), but the effect was not significant. Treatment for 22 days prevented the marked increase in nerve sorbitol found in the diabetic rats but did not prevent a fall in nerve myo-inositol. The results indicate that treatment with an aldose reductase inhibitor for a sufficient period of time can reduce the increased susceptibility of peripheral nerves to compression in streptozotocin-induced diabetes mellitus in the rat by a mechanism which may be related to the prevention of increases in sorbitol in the nerves.

Key words: Aldose reductase inhibitor, streptozotocin, axonal transport, compression syndromes, diabetes mellitus, mono-neuropathy, sorbitol, myo-inositol.

The peripheral nerves of the upper and lower extremity can become spontaneously compressed at different locations along their course in the limb. The most common syndrome in the upper extremity is the carpal tunnel syndrome where the median nerve is compressed in the carpal tunnel at the wrist. One of the diseases associated with carpal tunnel syndrome is diabetes mellitus and clinical studies have suggested that there is an increased susceptibility of peripheral nerves to trauma in that condition [1–5]. The clinical observations have been confirmed by experimental studies [6–9]. It is not known which factors are important for this increased susceptibility, but increased water content as a consequence of elevated levels of nerve sorbitol may be of

importance [9–11]. The present study was performed in order to investigate that possibility by treatment of the rats with an aldose reductase inhibitor (ARI), which reduces the accumulation of sorbitol and fructose in the nerve [9–12].

Materials and methods

Induction of diabetes

Weight-matched female Wistar rats, 11–14 weeks of age and weighing 170–250 g, were divided into five groups. Diabetes was induced in three groups with a single intravenous or intraperitoneal injection of streptozotocin (STZ; 50 mg/kg) dissolved in 0.01 mol/l citrate buffer, pH 4–4.5, in the same way as previously described [9, 15]. Two other groups of animals received an equivalent volume of citrate buffer (controls). Diabetic rats received no insulin. Fasted

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whole blood glucose was measured with glucose-sensitive reagent sticks in an Ames Glucochek at regular intervals after the STZ-injection. Only animals with whole blood glucose values above 14.0 mmol/l were included in the diabetic groups.

Experimental groups

The aldose reductase inhibitor, 'Statil' ICI 128 436 [3-(4-bromo-1-fluorobenzyl)-4-oxo-3H phthalazin-1-yl-acetic acid] was administered once daily orally by gavage at a dose of 25 mg/kg. 'Statil' is a trademark, the property of Imperial Chemical Industries PLC. Five groups of rats were established: (A) control rats treated with 'Statil' for 22 days; (B) control rats treated with 'Statil' for 7 days; (C) age-matched diabetic rats treated with 'Statil' from induction of diabetes; (D) age-matched diabetic rats treated with 'Statil' from Day 15 after streptozotocin for a further 7 days; (E) age-matched untreated diabetic rats.

Axonal transport

After 22 days of diabetes, the rats were anaesthetized with pentobarbital (Sagatal®; 60 mg/ml intraperitoneally) and the L₄ and L₅ spinal cord segments were exposed by a laminectomy of the L₁ and L₂ vertebrae as previously described [9, 15]. One hundred µCi of L-[4, 5] ³H-leucine (specific activity 124 Ci/mmol; Amersham, UK) were injected into the area of the motor neurone cell bodies through a glass micro-pipette according to the method described previously [9, 15]. Four to eight injections (total around 3 µl) were made into the left side of the spinal cord each time. The wound was sutured and animals were placed on insulating cushions under a heating lamp in order to keep body temperature constant at 37°–38°C. During anaesthesia intraperitoneal injections of isotonic saline were given to the diabetic rats to avoid dehydration (total 20–25 ml/rat).

Compression procedure

Four hours after the spinal cord injection, the left sciatic nerve was exposed in the thigh, with the rats again under anaesthesia. The nerve was carefully dissected free and a small compression chamber, consisting of two rubber membranes encased in perspex [9] was applied around the nerve trunk. The chamber was inflated with nitrogen to a pressure of 30 mmHg which was maintained for 3 h. The application of the chamber per se is known not to induce any inhibition of fast axonal transport [9, 16].

Determination of radioactivity

Three hours after application of the chamber the animals were killed and the sciatic nerve quickly dissected out and frozen. A loose suture

was placed around the nerve to indicate the position of the proximal edge of the compressed segment. Each nerve was cut into 2.5 mm pieces and the pieces were soaked individually in 2 ml of cold 10% trichloroacetic acid overnight at +4°C. The pieces were then washed once in a further 2 ml cold trichloroacetic acid and dissolved in 0.3 ml of Protosol® (New England Nuclear, Boston, Mass, USA) or 0.25 ml Optisolv either for 1 h at 55° or overnight at room temperature. Two ml of toluene-based scintillation fluid were added to each dissolved nerve piece and the radioactivity of each sample was measured in a liquid scintillation counter with automatic quench correction.

Determination of sorbitol and myoinositol

Sorbitol and myo-inositol were measured in the contralateral nerve by Gas-Liquid Chromatography according to the method of Stribling et al. [17] and expressed in terms of wet weight of nerve.

Statistical analysis

A ratio – the transport block ratio – reflecting the accumulation of transported radioactive proteins around the proximal edge of the compressed segment was calculated in the same way as previously described [9]. In short, the radioactivity in three nerve pieces (two pieces in the proximal part of the compressed area and one piece immediately proximal to the compressed area) was expressed as a fraction of the radioactivity contained in six pieces proximal to the above mentioned three pieces. Data were analysed for homogeneity of variance by the Bartlett-Box test and then by one-way analysis of variance. As our main interest lay a priori in the difference between untreated diabetic rats and diabetic rats treated with ARI, significance was calculated by least significant difference [18]. P-values < 0.05 were considered significant (two tail). The values are presented as mean ± SD, with 95% confidence limits where appropriate. Statistical comparison of nerve sorbitol and myo-inositol levels was by Student's t-test.

Results

Body weight and blood glucose

The changes in body weight and whole blood glucose levels during the experimental period are shown for all groups of rats in Table 1. The diabetic rats had whole blood glucose levels above 14 mmol/l, polyuria, polydipsia and weight loss; treatment with the ARI did not prevent weight loss or affect severity of diabetes.

Table 1. Changes in body weight and blood glucose concentration for all groups of rats

Treatment	Weight change (%)	Duration of diabetes (days ± 1 day)	Duration of treatment (days ± 1 day)	Blood glucose (mmol/l)
A ARI-treated non-diabetic rats (22 days); n = 5	+15.5 ± 6.2	—	22	—
B ARI-treated non-diabetic rats (7 days); n = 10	+ 6.7 ± 10.5	—	7	—
C ARI-treated diabetic rats (22 days); n = 6	–18.0 ± 6.4	22	22	19.9 ± 4.7
D ARI-treated diabetic rats (7 days); n = 8	–16.1 ± 6.4	14	—	22.3 ± 6.6
	–26.6 ± 4.2	22	7	> 14
E Untreated diabetic rats; n = 8	–21.4 ± 7.6	14	—	18.1 ± 3.5
	–19.6 ± 11.0	22	—	> 14

Body weights are mean ± SD of the % change between weights at 14 days or 22 days after induction of diabetes, or injection of citrate buffer as indicated, and weights prior to treatment. Blood glucose concentrations are mean ± SD. The apparent change in weight in group D between 14 and 22 days is not significantly different from that in group E

Table 2. Changes in nerve sorbitol and myo-inositol

Treatment	Sorbitol (nmol/mg wet weight)	Myo-inositol (nmol/mg wet weight)
A. ARI-treated non-diabetic rats (22 days); $n=5$	0.15 ± 0.12^a	4.17 ± 1.32^b
C. ARI-treated diabetic rats (22 days); $n=6$	0.33 ± 0.20^a	2.98 ± 0.42
E. Untreated diabetic rats; $n=8$	2.87 ± 0.87	2.75 ± 0.81

Values for nerve myo-inositol and sorbitol are means $\pm$ SD measured 22 days after induction of diabetes or injection of citrate buffer, in all groups. Where indicated, values were significantly different from those in the diabetic group at the $^a p < 0.001$ and $^b p < 0.05$ level

Fig. 1. Transport block ratio in control and diabetic nerves compressed at 30 mmHg for 3 h. Each bar is mean $\pm$ SEM of 5 to 10 experiments. A=non diabetic rats treated with ARI for 22 days. B=non-diabetic rats treated with ARI for 7 days. C=diabetic rats treated with ARI for 22 days. D=diabetic rats treated with ARI for 7 days. E=untreated diabetic rats. Groups marked* are significantly different from the group of diabetic rats at the $p < 0.05$ level

Nerve content of sorbitol and myo-inositol

The sciatic nerve content of sorbitol and myo-inositol for untreated diabetic rats and after 3 weeks of treatment of control rats and diabetic rats with ARI are summarised in Table 2. In untreated diabetic rats there was an approximately 10-fold accumulation of sorbitol and around 40% reduction of myo-inositol content as compared to treated controls. Treatment of diabetic rats with ARI maintained the content of sorbitol to near normal levels but did not prevent the myo-inositol deficit. This contrasts with other reports in which 'Statil' and other ARIs have been shown to protect against myo-inositol depletion [13, 19].

Inhibition of fast axonal transport

The transport block ratios in treated control rats, treated diabetic rats and untreated diabetic rats are summarised in Figure 1. Compression of the sciatic nerve at 30 mmHg for 3 h induced a slight accumulation of fast axonally transported proteins at the site of compression in control rats treated with the ARI. The transport block ratio (TBR) was 0.71 ± 0.17 ($n=10$) in rats treated with ARI for 7 days and 0.69 ± 0.08 ($n=5$) in rats

treated with ARI for 22 days. These values are not different from previously published data on untreated controls (0.67 ± 0.16 ; $n=7$) [9]. Compression, at the same pressure and for the same duration, of the sciatic nerve in untreated diabetic rats produced a marked inhibition of fast transport. In nerves of diabetic rats the transport block ratio was increased to 0.96 ± 0.24 ($n=8$), which is not different from previously published values for untreated diabetic rats (1.01 ± 0.35 ; $n=7$) [9]. The differences between *treated* controls and *untreated* diabetic rats were statistically significant. Compression at 30 mmHg for 3 h induced a less pronounced inhibition of fast transport in the sciatic nerve of *treated* diabetic rats as compared to *untreated* diabetic rats. The value for diabetic rats treated for 7 days was 0.85 ± 0.11 ($n=8$) and for diabetic rats treated for 22 days was 0.77 ± 0.12 ($n=6$). The latter was significantly different from the value for untreated diabetic rats; the difference in the values was 0.19 (95% confidence limits: 0.01 to 0.37).

The inhibition of fast axonal transport in the untreated diabetic rats was substantial but not complete; the transport block ratio lay approximately halfway between that in uncompressed nerves (around 0.5) and that in nerves which have been ligated (around 1.5), in which the distal part of all the axons will degenerate [9].

Discussion

The present study shows that local compression of rat sciatic nerves at 30 mmHg for 3 h induces a less pronounced accumulation of transported radioactive proteins in diabetic rats treated with an aldose reductase inhibitor than in untreated diabetic rats.

While only the rats treated with the ARI for the total duration of diabetes exhibited a significant prevention of the increased susceptibility to compression, the mean values of the TBR in the rats treated for 7 days after two weeks of diabetes was also less than that for untreated diabetic rats, suggesting that the ARI may be able to reverse as well as prevent the lesion, although a prolonged treatment period may be necessary to provide maximal benefit.

In the sorbitol pathway, which is present in many tissues including nerve, glucose is converted to sorbitol via aldose reductase; sorbitol dehydrogenase then converts sorbitol to fructose [20]. Nerve tissue can take up glucose in the absence of insulin and the sugar alcohols sorbitol and fructose cannot diffuse away from their site of synthesis. The accumulation of the sugar alcohols in diabetic nerves may generate osmotic fluid shifts [21] and the endoneurial space may expand due to an increased water content [10, 11]. The increased water content in diabetic nerves may affect the nerves by at least two different mechanisms either of which could increase the susceptibility to compression. First,

the nerve fibres have to take up more of the applied pressure than do axons in normal peripheral nerves where individual nerve fibres are protected by surrounding connective tissue giving structural support. Second, O₂ supply to axons may be compromised either by an increase in the diffusion distance of oxygen from endoneurial capillaries to axons through expansion of the endoneurial space, or by an effect on capillary blood flow. Endoneurial hypoxia has been shown to occur in diabetic neuropathy [22] and in galactose neuropathy in which a more pronounced endoneurial oedema is found [23–25]. An increased susceptibility to compression has been assumed to occur in galactose neuropathy [26].

Potent inhibitors of aldose reductase have become available during the last few years [27]. These inhibitors can completely prevent or reverse the accumulation of sorbitol and markedly reduce the accumulation of fructose in rat sciatic nerves as well as prevent the depletion of myo-inositol. This may lead to prevention or reversal of defects in axonal transport, and nerve conduction velocity deficit [13, 17, 19, 28–31]. It can also decrease the water content in diabetic nerves as indicated by measurement of wet weight/unit length of nerve [11, 32]. Treatment of galactose-intoxicated rats with an aldose reductase inhibitor restores to normal levels the elevated sodium concentration, the increased water content and interstitial fluid pressure characteristic of galactose neuropathy [33, 34].

In the present study, the elevated nerve content of sorbitol was also reduced by administration of Statil to the diabetic rats, although no significant restoration of myo-inositol was detected.

Since our experimental protocol did not include a diabetic control group treated with insulin it has to be emphasised that the slight increase in sorbitol levels, the increased transport block ratio as well as the fall in myo-inositol levels may have been due to extrapancreatic effects of streptozotocin (group C compared to group A).

The results indicate that increased susceptibility to nerve compression may be partly prevented by administration of an aldose reductase inhibitor for the duration of the diabetic state and, therefore, that the cause of increased susceptibility to compression is related to the increased content of nerve polyols.

The role of an impairment of fast axonal transport in nerve compression disorders is at present speculative; other features such as focal demyelination and axon dwindling need also to be taken into account. With regard to the latter, which is more readily equated with changes in slow transport, our own results indicate that compression levels which affect fast axonal transport similarly disrupt slow [35]. We would therefore predict that in the experiments described here, the inhibition of fast transport serves as a measure of effects on axonal transport in general. Aldose reductase inhibitors may reduce the impact which impairment of

axonal transport has on the course of nerve compression disorders, such as carpal tunnel syndrome in diabetic patients.

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