Prevention of Defective Axonal Transport in Streptozocin-diabetic Rats by Treatment with "Statil" (ICI 128436), an Aldose Reductase Inhibitor

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SUMMARY

This investigation examined the effect of treatment of streptozocin-diabetic rats with the aldose reductase inhibitor "Statil" (25 mg/kg/day p.o.) on axonal transport in cholinergic neurons of the sciatic and vagal nerves and on nerve polyol and sugar levels. Three weeks of experimental diabetes caused deficits in the accumulation of choline acetyltransferase activity proximal to 24-h constrictions in the left sciatic and vagus nerves. These deficits did not develop in age-matched, similarly diabetic rats that were treated with the aldose reductase inhibitor. The inhibitor prevented completely the build-up of sorbitol and markedly reduced the build-up of fructose in the sciatic nerves of the treated diabetic rats. The inhibitor also prevented the depletion of *myo*-inositol that was seen in the untreated diabetic animals. It is suggested that these findings indicate a possible approach to the elucidation of the pathogenesis of cardiac vagal dysfunction in diabetes mellitus.

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The aldose reductase inhibitors ICI 105552 and sorbinil prevent the development of a deficit of axonal transport of choline acetyltransferase activity in the sciatic nerves of rats with short-term experimental diabetes.[1,2] This deficit may be influential in the development of chronic diabetic neuropathy if it is indicative of a wider and progressive starvation of nerve terminals of vital components of axoplasm. To begin to assess this possibility and to examine further the significance of protection by aldose reductase inhibition, we designed the present study to extend observations to the parasympathetic preganglionic fibers of the vagus nerve. We have also examined an aldose reductase inhibitor, "Statil" [ICI 128436; 3-(4-bromo-2-fluorobenzyl)-4-oxo-3*H*-phthalazin-1-ylacetic acid],* which has not been studied previously in this context.

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MATERIALS AND METHODS

Experimental organization and treatment of rats. Three groups of rats were composed of age-matched male Wistars (350–370 g). Diabetes was induced in two groups with streptozocin (STZ) at 50 mg/kg i.p. after an overnight fast. On the same day, treatment of one of these two groups with Statil (25 mg/kg/day p.o.) was begun and maintained for 3 wk. On day 3, blood glucose was measured in the STZ-treated groups and rats with values <15 mmol/L were rejected. On day 20, each rat was anesthetized with halothane (5% in O_2 for induction; 1–2% in O_2 for maintenance) and tight prolene ligatures were applied to the left sciatic nerve (at mid-femur level) and the left vagus nerve (at laryngeal level). The rats were killed 24 h later, the left vagus and both sciatics removed and placed on ice-cold stainless steel plates for segmentation and assays.

Measurement of accumulation of choline acetyltransferase activity. The sciatic nerves were cut into 3-mm segments that were treated exactly as is described elsewhere.[2] In this way, a net accumulation of enzyme activity proximal to the constriction in the left sciatic nerve was calculated for each rat using the activity in contralateral segments of the unconstricted (right) nerve for background activity. The fiber distribution in the left and right vagi could not be assumed to be contralaterally similar. Our previous studies had demonstrated that ChAT activity accumulation in the left vagus proximal to a 24-h constriction was confined to the 4 mm of nerve trunk immediately adjacent to the ligature.[3] We also found that segments of nerve more proximal to this accumulation zone contained a level of activity that was similar to that measured in unconstricted left vagi.[3] Thus, we measured accumulation by subtraction of background activity, derived from that measured in the segment 4–8 mm proximal to the constriction, from the activity measured 0–4 mm proximal to

*"Statil" is a trademark; the property of Imperial Chemical Industries PLC.

TABLE 1
Effects of STZ diabetes of 3-wk duration, with and without daily aldose reductase inhibitor (ICI 128436), on the change in body weight (day 1 to day 21), terminal fasting blood glucose, and choline acetyltransferase activities accumulating proximal to 24-h ligatures and background levels in vagus and sciatic nerves

			Choline acetyltransferase activity (nmol ACh/h)			
			Net accumulation proximal to ligature		Background activity/mm nerve	
	Δ Body weight (g)	Blood glucose (mmol/L)	Sciatic	Vagus	Sciatic	Vagus
Controls, untreated (N = 12)	+34.9 ± 4.0	4.9 ± 0.5	4.73 ± 0.43 †	2.29 ± 0.14 *	2.91 ± 0.15 NS	0.64 ± 0.06 NS
Diabetics, untreated (N = 14)	−79.7 ± 5.1	18.6 ± 0.8	2.96 ± 0.33 *	1.76 ± 0.14 †	2.81 ± 0.17 NS	0.48 ± 0.06 NS
Diabetics, ICI 128436 (N = 10)	−66.1 ± 10.5	18.3 ± 0.9	4.16 ± 0.30	2.58 ± 0.20	3.01 ± 0.20	0.60 ± 0.06

Levels of significance by unpaired *t*-tests: *2P < 0.02; †2P < 0.005; NS, 2P > 0.05 (not significant).
Massive differences were not tested. The numbers of rats are in parentheses.

the constriction.[3] The same technique was used here except that the more proximal segment—assayed for background activity—was measured exactly but was not restricted to 4-mm length. This was done to maximize the number of replicates in the assay. Background activity was therefore expressed per millimeter of nerve (see Table 1).

Assay procedure. The methods employed for assay of blood glucose and of choline acetyltransferase activity are described in detail elsewhere.[2] No modifications of these procedures were introduced in the present study. The enzyme activity was measured as nanomoles acetyl choline (ACh) formed per hour and is expressed as such. Those portions of the unconstricted sciatic nerve surplus to requirements for choline acetyltransferase assay were weighed, extracted, and assayed for glucose, sorbitol, fructose and *myo*-inositol by gas chromatography of their silyl derivatives. Again, the procedures are described exactly elsewhere.[2]

RESULTS

Body weight and blood glucose. Over the 3-wk experimental period, both diabetic groups suffered marked weight loss in contrast to the slight weight gain in the control rats (Table 1). At death, both diabetic groups were markedly hyperglycemic (Table 1). Treatment with Statil was without significant effect on either of these variables.

Accumulation of choline acetyltransferase activity. Neither diabetes nor treatment with the aldose reductase inhibitor had a significant effect on the background activity of choline acetyltransferase in unconstricted sciatic nerve or left vagal segments that were proximal to and distant from the ligature (see Table 1, in which activities are presented per millimeter length of nerve). However, in both nerves the accumulations proximal to the ligatures were reduced significantly in the untreated diabetic rats by comparison with either the control (nondiabetic) rats or with the Statil-treated diabetic group (see Table 1 for levels of significance of difference). These latter two groups gave similar accumulations for both nerves.

Nerve sugar and polyol levels. These data are shown in Table 2. The glucose content of the sciatic nerves of both diabetic groups was markedly elevated by comparison with the nerves of the control rats.

Treatment with Statil prevented completely the build-up of sorbitol that was seen in the untreated diabetic rats. However, the fructose levels in the nerves of the treated rats were elevated well above controls, but were less than half those of the untreated diabetic rats. This shows that the inhibitor reduced substantially sorbitol pathway flux, but did not block the pathway completely.

The untreated diabetic rats showed a depletion of sciatic nerve *myo*-inositol. This deficit was prevented by treatment with the aldose reductase inhibitor.

DISCUSSION

This study shows that deficits in the accumulation of choline acetyltransferase activity proximal to ligatures applied to left

TABLE 2
Effects of STZ diabetes of 3-wk duration, with and without daily aldose reductase inhibitor, on sciatic nerve sugars and polyols

	Sciatic nerve contents (nmol/mg nerve)			
	Glucose	Sorbitol	Fructose	*myo*-Inositol
Controls, untreated (N = 12)	0.84 ± 0.25	0.17 ± 0.05	0.49 ± 0.13	3.54 ± 0.22 †
Diabetics, untreated (N = 14)	6.11 ± 0.48 NS	2.03 ± 0.28	5.95 ± 0.38	2.66 ± 0.18 *
Diabetics, ICI 128436 (N = 10)	7.64 ± 0.62	0.19 ± 0.04	2.20 ± 0.23	3.41 ± 0.18

Levels of significance by unpaired *t*-tests: *2P < 0.01; †2P < 0.005; NS, not significant at the 95% level.
Massive differences were not tested. The numbers of rats are in parentheses.

sciatic and vagus nerves of STZ-diabetic rats were prevented by treatment with the aldose reductase inhibitor, Statil. At the dose used (25 mg/kg/day) the inhibitor completely prevented sorbitol accumulation and substantially reduced the fructose levels in the sciatic nerves of the diabetic rats. The inhibitor also prevented depletion of sciatic nerve *myo*-inositol levels.

The effects on choline acetyltransferase activity accumulation proximal to nerve constrictions are consistent with the suggestion that the untreated diabetic rats showed a deficit in orthograde axonal transport of the enzyme and that this deficit was prevented by treatment with Statil. It might be expected that such a defect of axonal transport would result in reduced delivery of enzyme to the axon with a consequent fall in the level of activity assayed as "background" in the segments well proximal to the ligature (vagus) or in the segments of unconstricted sciatic nerve. Such a statistically significant difference was not present, although a trend in that direction was indicated, especially in the vagal segments of the untreated diabetic rats. The differences were small compared with the interanimal variation in enzyme activities. Furthermore, we do not know how much of the background activity is in the form of mobile enzyme, and the static fraction, if such exists, might not exhibit a reduction in activity until an axonal transport deficit had persisted for some time. Longer term studies are therefore needed to resolve this question.

The deficit in accumulation of enzyme in the constricted sciatic nerves of the untreated diabetic rats was similar in magnitude to those reported in earlier studies.[1-5] However, the only other study that examined the phenomenon in the vagus nerve failed to demonstrate a significant deficit in accumulation.[3] We cannot explain this, but the data of the present study indicate the vagal deficit to be well founded.

The effect of Statil on the axonal transport deficits agrees well with studies using a different but related compound,[1,5] and with studies using the structurally different sorbinil.[2] As with these earlier studies[1,2] and with those of others,[6-8] treatment with the aldose reductase inhibitor prevented depletion of nerve *myo*-inositol in the diabetic rats. The mechanism of this effect remains to be clarified, but the normalization of nerve sodium-potassium ATPase activity by sorbinil treatment of diabetic rats may serve to rectify a defect of sodium-dependent *myo*-inositol uptake in the nerve.[9]

We suggest that the major outcome of this study is the finding that the vagal axonal transport deficit was prevented by the aldose reductase inhibitor. Over the same time scale, STZ-diabetic rats develop abnormalities of conscious resting heart rate that may be a consequence of vagal dysfunction and that are also prevented by Statil treatment.[10] Indirect evidence suggests that loss of cardiac vagal control of heart rate in diabetic patients is a manifestation of dysfunction of the presynaptic, parasympathetic efferent neuron.[11] Extension of the work described here to diabetes of longer duration with tests of the functional competence of parasympathetic cardiac innervation might answer the following questions. First, whether experimentally diabetic rats exhibit vagal dysfunction similar to that seen in man; second, whether defective axonal transport contributes to the development of such a lesion; and third, whether aldose reductase inhibitors can offer protection against the development of such defects.

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