Deficient Axonal Transport of Substance P in Streptozocin-Induced Diabetic Rats

Effects of Sorbinil and Insulin

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This study measured the accumulation of substance P–like immunoreactivity (SPLI) proximal and distal to 12-h constricting ligatures applied to rat sciatic nerves. There were three separate experiments, and the baseline for each consisted of control and age-matched rats with 3 wk of untreated streptozocin-induced diabetes. We compared the effects of twice-daily insulin treatment, daily sorbinil (25 mg · kg^{-1} · day^{-1} p.o.), and a combination of both treatments. In untreated diabetic rats the anterograde accumulation of SPLI was reduced by 30–40%. This deficit was unaffected by sorbinil alone but was attenuated by insulin and prevented completely by insulin and sorbinil combined. There were also indications that diabetes caused reductions in retrograde accumulation of SPLI and its content in unconstricted nerve and the L4 dorsal root ganglion. The fraction of SPLI undergoing net anterograde or retrograde movement and the velocities of accumulation were unaffected by diabetes or the treatment regimens. These findings indicate a reduction in the amount of substance P moved by axonal transport in diabetic rats that is related partly to aldose reductase activity and partly to some other insulin-correctable consequence of experimental diabetes. *Diabetes* 37:488–93, 1988

Delivery of deficient amounts of essential components of axoplasm via anterograde axonal transport is becoming a more likely contributor to the etiology of degenerative distal neuropathies in diabetes mellitus. There is a substantial list of constituents of axoplasm that show axonal transport deficits in experimental diabetes (1,2). This study was designed to determine whether substance P should be added to that list. We had several reasons for its selection. The involvement of substance P in nociception (3) and gut motility (4) provides a rationale for its study in relation to disorders of these processes, which are present in many neuropathic diabetic patients (5–7). Recent work has shown that synthesis and axonal transport of substance P decline in nerves regenerating after an experimental lesion (8). Thus, the behavior of substance P may serve as an early indicator of degenerative and regenerative changes. Substance P is also a marker for a population of C-fibers; hence, we can be reasonably certain that we are studying transport in only one fiber population. Finally, we were able to use substance P to distinguish between deficits in the amount of material shifted by axonal transport and changes in the velocity at which it moves (MATERIALS AND METHODS). Hitherto this has not been possible for most of the defects described (1).

The aim was to determine whether abnormalities of substance P movement in sciatic nerves could be detected in rats with streptozocin-induced diabetes (STZ-D) of 3 wk duration. We also examined the effects of insulin and aldose reductase inhibition with sorbinil [(+)6-fluoro-spiro-[chroman-4,4′-imidazolidine]-2′,5′-dione; CP-45634, Pfizer, New York].

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MATERIALS AND METHODS

Experimental organization. This study comprised three virtually self-contained experiments. Each had a control group of nondiabetic rats age matched to two groups of diabetic rats. In each case, one of the diabetic groups comprised untreated rats, and the other underwent one of three treatment regimens. In the first experiment the treatment was twice-daily insulin, in the second the rats of the treated group were given the aldose reductase inhibitor sorbinil, and in the third experiment the experimental group was given a combination of both treatments.

Animals, induction of diabetes, and treatment. Male Wistar rats (weighing 290–330 g) were made diabetic by injection of 50 mg STZ/kg i.p., given in saline after an overnight

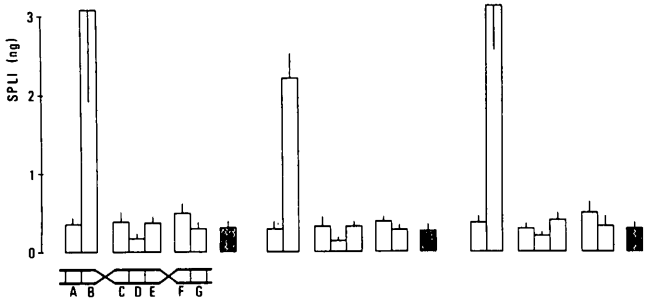

FIG. 1. Histograms showing distribution of substance P–like immunoreactivity (SPLI) in 5-mm segments of rat sciatic nerves constricted at 2 points 15 mm apart. *Left*, control rats; *center*, untreated diabetic rats; *right*, diabetic rats treated with insulin and sorbinil. *Shaded columns* represent background activity calculated as mean of *segments C–E*. Data are arithmetic means ± 1SD.

fast. Control rats were given saline alone. Three days after STZ was administered, a drop of blood obtained by tail prick was screened for glucose by a strip-operated reflectance meter (Reflomat; Boehringer Ingelheim, London). STZ-treated rats with blood glucose values of <15 mM were rejected from the study. In the designated groups, sorbinil treatment was begun on day 3 after STZ administration. The drug was administered, in distilled water, to conscious rats by gastric intubation via a 19-gauge syringe needle, the tip of which had been smoothed and rounded with silver solder, at a single daily dose of 25 mg/kg.

Three days after STZ injection, each rat designated for insulin treatment was given a starting dose of 2.5 IU/100 g body wt Semitard MC insulin (Novo, Copenhagen) on the morning of blood glucose measurement and 2.5 IU/100 g Monotard MC insulin (Novo) on the evening of that day. Thereafter, blood glucose was measured each morning, and animals with blood glucose values >9 mM were given a morning dose of 2 IU/g Semitard and an evening dose of 2 IU/100 g Monotard. Animals with a morning blood glucose value <9 mM were not injected with Semitard that morning but were given an evening dose of 2 IU/100 g Monotard provided their blood glucose level had risen to >9 mM. All insulin injections were given subcutaneously.

Nerve ligation and preparation for axonal transport studies. Anterograde and retrograde axonal transport of substance P–like immunoreactivity (SPLI) was studied by measurement of its accumulation proximal and distal to two synchronously applied ligatures tied around the femoral region of the sciatic nerve. Pilot studies showed that these accumulations were linear for at least 12 h.

Rats were anesthetized with halothane, and the right sciatic nerve was exposed. Two ligatures were placed around the nerve, the first positioned 10 mm distal to the gluteal branch and the second 15 mm distal to the first at midthigh level. The wound was closed with clips, and the animals were allowed to recover from the anesthetic.

After 12 h of survival, rats were killed by a blow to the head and exsanguination from the neck, and a blood sample was taken into a heparinized tube for blood glucose assay. Both sciatic nerves were removed rapidly. In some experiments the L4 dorsal root ganglia were also removed. The nerves were immediately frozen, under moderate tension, on a brass block precooled in liquid nitrogen. The constricted nerve was cut into seven 5-mm segments. Three 5-mm segments were taken from the equivalent region of the contralateral nerve for determination of SPLI. The remainder of this nerve was stored for estimation of polyols and sugars. All segments were placed in 1-ml plastic tubes that had been precooled in liquid nitrogen to prevent the tissue from thawing on contact with the tube. The segments were stored in liquid nitrogen and processed later the same day.

Nerve polyols and blood glucose estimation. The remaining portion of the unconstricted nerve was stored at −80°C until assay of trimethylsilyl derivatives of nerve glucose, fructose, sorbitol, and *myo*-inositol concentrations was performed by gas chromatography (9).

The blood samples taken at death were centrifuged (1 min, 9000 × *g*), and the plasma glucose concentrations were determined by a hydrogen peroxidase–sensitive static-phase glucose analyzer (YSI Model 23AM, Yellow Springs, OH). Whole-blood glucose values were calculated by multiplying the plasma value by a factor of 0.89, which corrected for red blood cell mass with respect to hematocrit.

Substance P extraction. The frozen nerve segments and dorsal root ganglia were transferred from the tubes stored in liquid nitrogen into 1-ml plastic tubes containing 200 μl boiling extraction buffer (2 M glacial acetic acid, 10 mM HCl, 1 mM disodium EDTA, and 1 mM dithiothreitol). Transfer was rapid to avoid thawing, which may have caused a rapid and selective loss of a substance P pool (10). The samples were boiled for 10 min and then homogenized with hand-held Teflon pestles. Pestles were rinsed with another 200 μl of buffer, to give a final volume of 400 μl. The homogenates were centrifuged (9000 × *g*) for 5 min at room temperature. The supernatants, containing the extracted substance P, were transferred to clean tubes and freeze-dried.

Radioimmunoassay of substance P. Assays were carried out as previously described with rabbit antiserum prepared against synthetic substance P conjugated to succinylated thyroglobulin and used at a final dilution of 1:210,000 (vol/vol). The characteristics of this antiserum have been described elsewhere (11). ^{125}I-labeled Tyr^{8}–substance P was used as the tracer. Samples were reconstituted in barbitone buffer (11,12) and assayed in triplicate. A standard curve was constructed for each assay to determine SPLI of the samples by interpolation.

Calculation of indices of axonal transport. The ligated sciatic nerve was divided into seven 5-mm segments. Two segments were taken proximal to the first ligature and distal from the second ligature. The isolated section of nerve between the ligatures was divided into three segments; one segment of 5 mm was cut from each end of the isolated nerve length, leaving a central segment of 4–6 mm that was measured to the nearest 0.5 mm. SPLI content of this segment was corrected to SPLI content per 5-mm segment.

Figure 1 shows the SPLI content of each segment in the constricted nerves for one experiment. Migration of SPLI away from the cell bodies (anterograde transport) caused accumulation proximal to the first (upper) ligature (*segment B*). Retrograde transport from the periphery resulted in a smaller accumulation of SPLI distal to the second (lower) ligature (*segment F*). In the portion of nerve between the ligatures there was a redistribution of SPLI to give smaller accumulations via anterograde and retrograde transport

(*segments E* and *C*, respectively). This redistribution of SPLI within the isolated portion of nerve was complete 6 h after constriction. Thus, the SPLI in *segment D* represents material that was probably in steady-state bidirectional flux; i.e., there was no longer any net movement of SPLI into or out of this segment. This is sometimes referred to as the *nonmobile fraction*, with reference to the total SPLI present in a segment of unconstricted nerve of equal length (13–15). This term is inaccurate but is, perhaps, convenient. The residual fraction, i.e., the mobile fraction, gives the amount of SPLI that is subject to net shift. In our experiments, this was calculated as

$$\text{Mobile fraction} = \frac{(\text{SPLI of isolated segment/mm}) - (\text{SPLI of segment D/mm})}{\text{SPLI of isolated segment/mm}}$$

The level of SPLI per unit length of nerve in the unconstricted segments was consistently higher than the SPLI content per unit length of the isolated segment ($P < .01$, paired *t* test). This result suggests that isolation of a portion of nerve between two ligatures causes a net loss in its content of SPLI. This invalidates the use of SPLI content of the unligated nerve as a measure of background activity, which is required for determination of accumulations of SPLI adjacent to the ligatures in the constricted nerve. Thus, the mean SPLI content of the isolated section of ligated nerve was taken as a measure of background SPLI, and this value was used in calculations of net accumulation and rate of accumulation. Comparison, by paired *t* test, of SPLI in *segment A*, with the mean SPLI per 5 mm of the isolated segments (*C–E*), showed no difference; therefore, the proximal accumulation was judged to be restricted to *segment B*, immediately proximal to the first ligature, and net accumulation was calculated as SPLI content of *segment B* minus the mean SPLI content per 5 mm of *segments C–E*. The rate of anterograde accumulation was calculated, after the fashion described originally by Partlow et al. (16)

$$\text{velocity (mm/h)} = \frac{\text{net accumulation (pg)}}{\text{mobile fraction} \times \text{background (pg)}} \times \frac{\text{segment length (mm)}}{\text{ligation time (h)}}$$

Net accumulation of retrogradely transported material was calculated as the SPLI content of *segment F* immediately distal to the second ligature minus background SPLI. Comparison by paired *t* test of background SPLI with SPLI content of *segment G* showed no differences. Rate of retrograde accumulation was calculated from an equation analogous to the one cited above.

Statistical analysis. All values are given as means ± SD. Comparisons between groups within experiments were made by one-way analysis of variance (ANOVA) with the Statistical Package for Social Scientists (SPSS-X, Chicago, IL) run on an ICL 2900 computer. Statistical significance was accepted when the *F* test gave $P < .05$. Group means were compared by Duncan's multiple-range tests at $P < .05$ and $< .01$. No comparisons were made between experiments.

RESULTS

Body weight and glycemia. Untreated diabetic rats of all three experiments lost weight and were hyperglycemic at death (Table 1). The three groups of untreated diabetic rats differed markedly in the severity of their hyperglycemia at death, despite parity in weight loss. We therefore believe that the blood glucose levels must reflect the timing of blood sampling in relation to eating patterns rather than differences among groups in the severity of diabetes. Sorbinil treatment did not affect body weight or final blood glucose.

Although the blood glucose levels in the insulin-treated

TABLE 1
Effects of 3-wk streptozocin-induced diabetes on body weight change, final blood glucose, and nerve polyols and sugars

Rats	*n*	Body weight (g)		Final blood glucose (mM)	Sciatic nerve content (nmol/mg wet wt)			
		At start	At death		Glucose	Sorbitol	Fructose	*myo*-Inositol
Experiment 1								
Control	8	302 ± 5	317 ± 17*	6.4 ± 0.6†	2.02 ± 1.03†	0.41 ± 0.19†	1.85 ± 0.90†	5.77 ± 0.91*
Untreated diabetic	9	310 ± 9	257 ± 23†	28.7 ± 5.5‡	11.20 ± 2.40‡	3.66 ± 0.76‡	9.67 ± 1.4‡	3.79 ± 0.29†
Insulin-treated diabetic	10	309 ± 8	314 ± 15*	20.2 ± 2.9*	7.76 ± 1.64*	2.18 ± 0.52*	5.58 ± 1.2*	5.29 ± 0.91*
Experiment 2								
Control	4	314 ± 11	346 ± 6†	7.2 ± 1.1†	1.33 ± 0.39†	0.30 ± 0.12†	0.66 ± 0.08†	4.53 ± 0.11†
Untreated diabetic	9	303 ± 7	243 ± 28*	50.4 ± 6.0*	13.25 ± 1.28*	3.53 ± 0.68*	7.78 ± 1.0‡	3.34 ± 0.42*
Sorbinil-treated diabetic	9	311 ± 7	247 ± 24*	51.0 ± 7.9*	15.27 ± 2.96*	0.62 ± 0.59†	2.87 ± 1.3*	4.58 ± 0.27†
Experiment 3								
Control	9	307 ± 8	314 ± 11*	6.4 ± 0.8†	1.00 ± 0.28†	0.24 ± 0.10*	1.06 ± 0.19*	5.15 ± 0.50*
Untreated diabetic	10	306 ± 11	250 ± 23†	34.2 ± 8.1‡	12.10 ± 3.50‡	4.25 ± 0.98†	10.70 ± 2.10†	3.59 ± 0.27†
Insulin- and sorbinil-treated diabetic	12	311 ± 9	303 ± 9*	22.3 ± 12.1*	6.4 ± 4.2*	0.17 ± 0.20*	0.68 ± 0.65*	5.52 ± 0.54*

Data are arithmetic means ± 1SD. Data from each experiment were analyzed by one-way ANOVA, and where *F* tests gave $P < .05$, group means were compared by Duncan's range tests. Level of significance was $P < .01$ for * vs. †, * vs. ‡, and † vs. ‡. No other differences were significant. No comparisons were made between experiments.

TABLE 2
Effects of streptozocin-induced diabetes in rats, with and without insulin treatment, on axonal transport of substance P–like immunoreactivity (SPLI) in doubly constricted sciatic nerves and on the content of SPLI in the contralateral unconstricted sciatic nerve

		Anterograde transport			Retrograde transport		
Rats	*n*	Accumulation proximal to upper ligature (pg)	Rate of accumulation (mm/h)	Mobile fraction	Accumulation distal to lower ligature (pg)	Rate of accumulation (mm/h)	Content of unconstricted nerve (pg/cm)
Control	8	2173 ± 637*	8.0 ± 1.5	0.48 ± 0.10	141 ± 68	0.74 ± 0.35	666 ± 252
Untreated diabetic	9	1482 ± 352†	6.3 ± 3.9	0.50 ± 0.12	66 ± 43	0.47 ± 0.51	570 ± 166
Insulin-treated diabetic	10	1735 ± 552	6.3 ± 2.9	0.52 ± 0.16	162 ± 112	0.81 ± 0.74	606 ± 140

Data are arithmetic means ± 1SD. Data were analyzed by one-way ANOVA, and where F tests gave $P < .05$, group means were compared by Duncan's range tests. This gave $P < .05$ for * vs. †. No other differences were significant.

diabetic rats were within the hyperglycemic range at death, we must stress that the rats were given half-doses of insulin in their final 24 h to obviate hypoglycemia during the postoperative period. Clearly, therefore, the final blood glucose levels bear no real relation to the levels that must have pertained during the experiment. We were disinclined to attempt to assess control on the basis of daily blood glucose measurements because these samples were taken before morning insulin injections and did not reflect the degree of control achieved. In contrast, we argue that the body weights give a better indication of the efficacy of the insulin management. In both experiments involving insulin the treated diabetic rats died at body weights similar to those of their nondiabetic controls.

Nerve polyol and sugar contents. The nerves of the untreated diabetic rats contained elevated levels of glucose, sorbitol, and fructose (Table 1). Nerve glucose was significantly reduced in both insulin-treated groups, though levels were significantly higher than those in control nerves. Insulin treatment also reduced the nerve sorbitol and fructose levels. Sorbinil alone reduced nerve fructose levels and completely prevented sorbitol buildup. When given together, the two treatments resulted in nerve levels of sorbitol and fructose that were virtually identical to those of control rats.

Nerve *myo*-inositol was depleted in the untreated diabetic rats of all three experiments. This effect was prevented by both sorbinil and insulin and by the two in combination.

Axonal transport of SPLI. The distribution of SPLI in the segments of constricted sciatic nerves is shown for one of the experiments in Fig. 1. The pattern of distribution within the nerves was similar in all experiments. There was a marked accumulation of SPLI immediately proximal to the upper ligature (*segment B*), which we attributed to interruption of anterograde axonal transport. There was also an accumulation distal from the lower ligature (*segment F*) due to interruption of retrograde axonal transport. There was a redistribution of SPLI in the nerve isolated between the ligatures, giving smaller accumulations in *segments E* and *C*. In the untreated diabetic group the SPLI content of all segments showed a numerical reduction, and in the case of the treated group shown in Fig. 1, this effect was prevented by the treatment.

In all three groups of untreated diabetic rats the accumulation of SPLI at sciatic nerve ligatures was reduced compared with the matched control groups (Tables 2–4). In diabetic rats, accumulations proximal to the upper ligature were 60, 68, and 72% of respective control groups. The changes were significant in two of the three cases (see tables for P values). Accumulations distal to the lower ligature were reduced in the untreated diabetic rats to 47, 48, and 62% of respective control values. None of these changes attained significance by the method used, but the presence of a substantial numerical reduction in all three experiments perhaps indicates a trend toward impaired retrograde accumulation. Similarly, there was a numerical reduction in the content of SPLI per unit length unconstricted nerve in the untreated diabetic groups compared with control rats, which was not significant as tested.

TABLE 3
Effects of streptozocin-induced diabetes in rats, with and without sorbinil treatment, on axonal transport of substance P–like immunoreactivity (SPLI) in doubly constricted sciatic nerves and on the content of SPLI in the contralateral unconstricted sciatic nerve

		Anterograde transport			Retrograde transport		
Rats	*n*	Accumulation proximal to upper ligature (pg)	Rate of accumulation (mm/h)	Mobile fraction	Accumulation distal to lower ligature (pg)	Rate of accumulation (mm/h)	Content of unconstricted nerve (pg/cm)
Control	4	2611 ± 880*	11.9 ± 6.3	0.52 ± 0.17	167 ± 150	0.89 ± 1.00	468 ± 128
Untreated diabetic	9	1554 ± 513†	8.1 ± 2.3	0.50 ± 0.12	80 ± 53	0.41 ± 0.28	422 ± 102
Sorbinil-treated diabetic	9	1527 ± 472†	12.3 ± 5.7	0.42 ± 0.29	87 ± 39	0.69 ± 0.67	442 ± 138

Data are arithmetic means ± 1SD. Data were analyzed by one-way ANOVA, and where F tests gave $P < .05$, group means were compared by Duncan's range tests. This gave $P < .01$ for * vs. †. No other differences were significant.

TABLE 4
Effects of streptozocin-induced diabetes in rats, with and without treatment with sorbinil and insulin, on axonal transport of substance P–like immunoreactivity (SPLI) in doubly constricted sciatic nerves and on the content of SPLI in the contralateral unconstricted sciatic nerve

		Anterograde transport			Retrograde transport		
Rats	*n*	Accumulation proximal to upper ligature (pg)	Rate of accumulation (mm/h)	Mobile fraction	Accumulation distal to lower ligature (pg)	Rate of accumulation (mm/h)	Content of unconstricted nerve (pg/cm)
Control	9	2740 ± 1285	8.6 ± 3.0	0.49 ± 0.07	199 ± 128	0.71 ± 0.50	662 ± 212
Untreated diabetic	10	1960 ± 450*	8.6 ± 4.2	0.44 ± 0.09	123 ± 51	0.57 ± 0.41	592 ± 132*
Insulin- and sorbinil-treated diabetic	12	2824 ± 728†	12.1 ± 5.4	0.39 ± 0.11	225 ± 157	1.23 ± 1.15	800 ± 208†

Data are arithmetic means ± 1SD. Data were analyzed by one-way ANOVA, and where F tests gave $P < .05$, group means were compared by Duncan's range tests. This gave $P < .01$ for * vs. †. No other differences were significant.

Treatment with insulin alone achieved a small increase in the level seen in untreated diabetic rats in anterograde accumulation and gave a retrograde accumulation that was numerically similar to that of the control rats (Table 2). Neither value differed significantly from the corresponding datum in control rats. Treatment with sorbinil alone was without effect on either anterograde or retrograde accumulations of SPLI (Table 3). When the two treatments were given in combination, the anterograde accumulation in the nerves of the treated diabetic group was numerically similar to that in the controls and was significantly higher than the value measured in the nerves of the untreated diabetic rats.

Calculation of the mobile fraction and rates of accumulation of SPLI gave no indications of an effect of diabetes or of treatment. Thus, mobile fraction was similar in all nine groups of rats, and although there was some variation in anterograde and retrograde rates of accumulation, there were no systematic or significant changes in any diabetic groups.

SPLI in dorsal root ganglia. In experiment 3 (involving treatment with insulin and sorbinil) the SPLI content of the L4 dorsal root ganglion contralateral to the constricted nerve was measured. The content (pg/ganglion ± 1SD) was lower in the ganglia from untreated diabetic rats (440 ± 139) compared with control rats (535 ± 186). The ganglia from the treated rats had SPLI levels (539 ± 190) similar to those of the control rats. None of the differences was significant by ANOVA.

DISCUSSION

Values for the SPLI content of any given nerve segment were subject to large coefficients of variation. We cannot state categorically whether this result reflects biological variation or variation in the efficacy of extraction and measurement. Our assay triplicates agreed (<5% variation) in most cases. There was also agreement between the measured SPLI contents of consecutive segments of unconstricted nerves, which had been subject to separate extractions. We are therefore inclined to suspect that a large part of the variation is of biological origin.

This background of variation meant that only large changes in SPLI attained statistical significance by the commonly accepted norms. We even failed to establish a significant difference in anterograde accumulation of SPLI between one pair of control and untreated diabetic groups; this was attributable to an inordinately large degree of variation in the control group. However, within this experiment there was a significant difference in the anterograde accumulations of SPLI between the group treated with insulin and sorbinil and the untreated diabetic group. Within the other two experiments there were significant reductions in anterograde accumulations in the untreated diabetic groups compared with their controls. We therefore do not hesitate in claiming that 3 wk of untreated STZ-D was associated with a significant reduction in the accumulation of SPLI proximal to a ligature that arrested anterograde transport for 12 h.

Numerical differences between control and untreated diabetic rats in retrograde accumulation of SPLI were larger than those in anterograde accumulation, but these were not significant as tested. This result is attributable to the much smaller magnitude of the retrograde accumulation, relative to the variation in the SPLI content of *segments C–E* (background) and *F*, in which the accumulation occurred. It is, however, reasonable to suggest that a pattern emerges, even in the absence of statistically significant differences. In the untreated diabetic rats of all three experiments there were also numerical reductions in background SPLI, unconstricted nerve SPLI, and SPLI accumulated distal to the lower ligature. The SPLI content of the L4 dorsal root ganglia of the untreated diabetic rats was also numerically, but not significantly, reduced in experiment 3. In contrast, there were no indications of any systematic changes in the rates of accumulation of SPLI or in its mobile fraction. Thus we believe that the accumulation deficit in the nerves of untreated diabetic rats did not indicate a flaw in the axonal transport process per se. The theoretical alternatives are defective loading of substance P onto the axonal-transport process or impaired synthesis of substance P. A fault in the loading mechanisms with normal synthesis would be expected to cause an increase in the SPLI content of the dorsal root ganglia. Clearly, this did not occur, so instead we believe that our findings indicate reduced ganglionic synthesis of substance P.

Such a possibility is supported by the earlier finding that there is reduced ganglionic output of proteins forming the slow component of anterograde axonal transport, a phenomenon that is prevented by insulin (17). If reduced protein synthesis does occur, it might be secondary to reduced

amino acid uptake (18). Thus, one hypothesis might be that some basic biochemical defect, perhaps linked to membrane Na^+-K^+-ATPase (19), could lead to a general deficit in cell body protein synthesis, with the result that various components of axoplasm, including substance P, might suffer reduced output via anterograde axonal transport.

There is an alternative possibility, i.e., that the changes in SPLI reflect a response of the cell body to axonal degeneration. There is little doubt that chemically induced diabetes in the rat causes axonal degeneration accompanied by ultrastructural signs of sprouting and regeneration (20–22). These changes are said to be less marked in insulin-treated diabetic rats (23). Ultrastructural evaluation of these phenomena has been reported in rats made diabetic for months rather than weeks; however, it is likely that biochemical changes in the nerves associated with degeneration/regeneration would precede detection of such changes by electron microscopy. Recent work has shown that the behavior of axonally transported SPLI is a sensitive indicator of change in nerves induced to undergo degeneration/regeneration in response to axotomy (8). In the regenerating axon the output of anterogradely transported transmitter substances—of which substance P is a putative example in C-fibers (24)—is reduced, presumably in favor of an increased output of materials necessary for regeneration (8). It is therefore possible that the changes reported in our study represent an early manifestation of cell body changes associated with regeneration in diabetes. Retrograde transport of nerve growth factor (NGF) is known to increase the substance P content of recipient dorsal root ganglia (25). Delivery of NGF via retrograde transport may be a necessary prerequisite for the maintenance of output of substance P via anterograde axonal transport. Retrograde transport of NGF is impaired in sciatic nerves of rats with STZ-D of a duration similar to that used in our study; indeed, delivery to the dorsal root ganglion was shown directly to be reduced in the diabetic rats (26). It is therefore possible that we are seeing changes in SPLI as one link in a chain of events, begun by the factors that limit retrograde transport of NGF and culminating in attempts by the nerve cell body to respond to a perceived axonopathy.

The effects of our treatment regimens were interesting. There is no doubt that treatment with sorbinil caused an effective inhibition of aldose reductase; the nerve sorbitol and fructose levels (Table 1) showed this clearly. Thus, the failure of sorbinil, when given alone, to make any impact on the SPLI accumulation deficit implies, at first sight, that aldose reductase is not involved in the development of the defect. However, the amplification of the effect of insulin by sorbinil should indicate the opposite. Indeed, with respect to the potential future of aldose reductase inhibitors in the management of diabetic complications, it is more relevant to assess their potential against a background of partially effective insulin therapy than to give the drugs in isolation. On that basis we argue that our findings indicate a partial involvement of aldose reductase in the development of deficits of axonally transported substance P in the sciatic nerves of STZ-D rats.

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