Resistance to axonal degeneration after nerve compression in experimental diabetes

(fiber injury/streptozotocin-induced diabetes/nerve myo-inositol, sorbitol, and fructose)

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Communicated by Ralph T. Holman, December 19, 1988 (received for review August 8, 1988)

ABSTRACT To determine the effect of diabetes on the development of axonal degeneration after acute nerve compression, the mobilized peroneal nerves of rats with streptozotocin-induced diabetes and of control rats were compressed at 150 mmHg (1 mmHg = 133 Pa) for 30 min by using specially devised cuffs. At three intervals after compression—3 days, rats diabetic for 31 wk; 14 days, diabetic for 6 wk; and 24 days, diabetic for 31 wk—groups of nerves were studied to assess numbers and sizes of fibers above, at, and below the cuff and to assess frequency of fiber degeneration in teased fibers from nerve distal to the cuff. Teased fibers with pathologic abnormalities were more frequent in nerves from controls than in nerves from diabetic rats in all groups but the difference was statistically significant only at 3 and 14 days after compression. The lack of significant difference at 24 days may be explained by higher rates of disappearance of degenerating products and of fiber regeneration at 24 than at 3 and 14 days. This study provides evidence that in addition to delaying the reported functional deficit of vibratory detection threshold and conduction block during nerve compression, diabetes also may partially prevent axonal injury. Low nerve myo-inositol concentration did not predispose diabetic nerve to acute compression injury. If these results also apply to human diabetes and if repeated acute compression is involved in the genesis of fiber degeneration in entrapment, then a higher frequency of entrapment neuropathy among diabetics might be due to mechanisms other than increased susceptibility of fibers to acute compression—e.g., possibly to greater constriction of nerve due to pathologic alterations of the carpal ligament.

Chronic entrapment neuropathies, such as the carpal tunnel syndrome, are said to be more common among diabetics (1, 2) than among nondiabetics. An epidemiologic population-based review of medical records in Olmsted County seems to indicate that the carpal tunnel syndrome is two to three times more frequent in diabetics than it is in the general population (J. C. Stevens, personal communication). Assuming entrapment neuropathy to be more common in diabetics, excessive fiber vulnerability, decreased reparative capacity, or increased external constriction may be involved. On the other hand, vibratory perception (3, 4) and nerve conduction (5, 6) fail earlier during cuff compression in controls than in diabetics, suggesting that diabetes inhibits the development of functional deficits.

The present studies assess whether standard cuff compressions of the peroneal nerve cause a significantly lower frequency of fiber degeneration in controls than in streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Cuff Design. The cuff used in study 1 consisted of two balloons each fitted over rectangular box-like metal frames with the open side (bottom or top) of each frame facing the nerve (Fig. 1). The necks of the balloons were tied and glued to a short tube that was soldered into the sides of each frame connecting an aneroid manometer with the inside of the frame and balloon. The inner surfaces of the balloons were glued to the outer surfaces of the sides and closed (top or bottom) side of the metal frames facing away from the nerve. At various times (see Results) after the rats became diabetic the deflated balloons and frames were positioned so that one balloon and its frame were under a mobilized 1-cm segment of the peroneal nerve and the other balloon and frame were over it. When the two opposing balloons were inflated, the nerve was squeezed between them. The pressure used in an experiment was the pressure desired on the nerve plus the pressure needed to just appose the balloons in air for the width of the cuff.

In study 2, the balloons were made of nondistensible silastic sheeting with subdermal implants (no. 501-1, Dow) (cuff 2), but in other respects the cuff was exactly like the first cuff. The balloons were fashioned so that when just inflated but at 0 pressure, their apposing surfaces would just meet for the width of the cuff. The pressure exerted on the nerve therefore should be the same as that recorded on the manometer.

Both cuffs were 8 mm wide.

Histologic Processing. At 3, 14, and 24 days after cuff compression [150 mmHg (1 mmHg = 133 Pa) for 30 min], the peroneal nerve was fixed in situ for 15 min with 4% glutaraldehyde in 0.025 M sodium cacodylate buffer at pH 7.38 (body temperature). After fixation, the nerve was removed and immersed in 2.5% glutaraldehyde in buffer for 24 hr for epoxy embedding and for 1 hr for teased fiber preparation. The further histologic processing and method of teasing to provide representative samples were as outlined elsewhere (7).

Morphometric and Teased Fiber Assessment. Transverse 0.75-μm epoxy-embedded sections of the fixed nerves were stained with phenylenediamine, and the number of myelinated fibers (MFs) per nerve and the size distribution of MF diameters (diameter of a circle of equivalent area) were obtained by using previously described techniques and our imaging system for nerve morphometry (ISNM) (7). MF profiles considered to represent degenerating fibers were not included.

One hundred teased fibers from each peroneal nerve were graded according to our published criteria (7): A = normal; B = myelin irregularities; C = demyelination; D = demyelination.

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Mobilized peroneal nerve of rat was compressed between two inflated balloons, one positioned beneath and the other above the nerve. The cuffs are balloons (solid lines and hatching) held in place by underlying metal frames (broken lines) whose edges in the inflated position did not contact nerve. For study 1, the balloon was Silastic; for study 2, an improved design was used (see text).

Studies. Study 1. Male 250-g Sprague–Dawley rats were ear tagged and randomly assigned to diabetic and control groups. Diabetes was present (fasting plasma glucose > 16 mmol/liter) by the third day after an intraperitoneal injection of streptozotocin (60 mg/kg). Controls were pair-fed.

Under pentobarbital anesthesia, peroneal nerves were exposed by blunt dissection without interruption of the blood supply and were undercut for a distance of 1 cm. The cuff was positioned, inflated to 150 mmHg for 30 min, and then deflated. The nerve was kept moist with Ringer’s saline. The wound was closed by surgical clips. The same procedure then was performed on the contralateral side. On day 4, the left wound was reopened, and the peroneal nerve was exposed and fixed in situ with glutaraldehyde. For removal of the nerve, this limb was amputated at the upper thigh, because in situ fixation prevented wound healing. Collars of nerve tissue were taken from the nerve above, at, and below the cuff, osmicated, and embedded in epoxy. An 8-mm length of nerve distal to the distal tissue collar was used for the preparation of teased fibers. On the 25th day, the right peroneal nerve was fixed and processed by the same methods. Four nerves were used from each of the 3-day and 24-day diabetic groups and six nerves were used from each of the 3-day and 24-day control groups.

Study 2. The second study was performed because (i) the results that were obtained in study 1 needed confirmation based on a larger study, (ii) an improved cuff was designed to deliver more exact pressures, and (iii) glucose, fructose, sorbitol, and myo-inositol concentrations were to be studied.

The selection and feeding of groups, the position of the cuff, the pressure and duration of cuff compression, and the methods of nerve processing were like those in study 1. The compression was performed at 6 wk after induction of the diabetic state, and the tissue was harvested 14 days later. The right unfixed peroneal nerve was frozen in isopentane at −80°C until analysis, and the endoneurial glucose, fructose, sorbitol, and myo-inositol concentrations were measured as described (8).

RESULTS

Study 1. The rats had been diabetic for 31 wk before the nerves were compressed.

Experiment 1: 3 days after cuff compression. Teased Fibers. Many teased fibers were undergoing changes typical, but not diagnostic, of early axonal degeneration. The frequency (mean ± SD) of all teased fiber abnormalities, mainly myelin irregularities (condition B) and paranodal demyelination (condition C), was significantly higher in controls (27.1% ± 12.4%) than in diabetics (8.0% ± 4.3%; 0.025 > P > 0.01) (Fig. 2).

Morphometry. The numbers of MFs per nerve were not significantly different between diabetics and controls above, at, and below the cutoff. MF diameter and ln(axonal area) were smaller in the diabetic nerves than in control nerves. These decreases cannot be attributed to the compression injury because they also were found in proximal nerve and are typical of nerve in untreated streptozotocin-induced diabetes (9, 10). For this reason, we compared differences between controls and diabetics in MF diameter, ln(axonal area), and ln(myelin area) at and distal to the cuff relative to values proximal to it at the 2.5th, 50th, and 97.5th percentiles. No statistically significant difference was found (table on request).

Experiment 2: 24 days after cuff compression. Teased Fibers. The frequency (mean ± SD) of all abnormalities of teased fibers was higher in controls (42.7% ± 11.7%) than in diabetics (34.7% ± 19.5%), but this difference was not statistically significant (P > 0.05).

Morphometry. No statistically significant differences between diabetics and controls were found for MF diameter, ln(axonal area), or ln(myelin area).

Study 2. The mean body weights of the diabetic and control rats were not significantly different at the time of sacrifice;
the rats had lost ≈50 g in weight in both groups since the induction of diabetes. Mean fasting plasma glucose was 4.3 mmol/liter in controls and 19.8 mmol/liter in diabetics ($P < 0.001$). There was a manyfold increase of endoneurial glucose, fructose, and sorbitol in the endoneurium of diabetic nerves ($P < 0.001$, Fig. 3). By contrast, endoneurial myo-inositol was decreased to less than one-half of that in controls ($P < 0.015$, Fig. 3).

Teased Fibers. The frequency of abnormality was significantly higher in controls (38.2% ± 13.4%) than in diabetics (25.4% ± 10.1%, 0.01 < $P < 0.025$) (Fig. 2). Axonal degeneration was almost the only pathologic abnormality encountered. Less than 1% of the abnormalities were demyelination or remyelination changes.

Morphometry. In previous studies we have established that the number of MFs in peroneal nerve of control rats is not significantly different between the proximal and distal (to the cuff) levels but that the fiber diameters are slightly smaller, due to tapering, in the distal level (data on request). Streptozotocin diabetes in rats does not induce a change in number of MFs per nerve but prevents development or reduces axon caliber (9). Direct comparison of numbers and size distribution of MFs per nerve in the distal segment of controls and diabetics might reflect loss of fibers from degeneration or gain of fibers from regeneration. The diabetic condition might affect the size of fibers. To minimize the effect of the diabetic condition, we normalized the morphometric results of distal nerve to that of proximal nerve. Rate of fiber degeneration was estimated separately by analysis of teased fiber conditions (previous section).

The mean transverse fascicular area of the distal nerve was increased by ≈70% in controls and by ≈30% in diabetics ($P < 0.001$) relative to the proximal nerve (Fig. 4). For controls and diabetics, the number of MFs per nerve was ≈20% less distal to the cuff than proximal to it. The difference in number of MFs per nerve of distal from proximal nerve was greater in controls than in diabetics but this did not reach statistical significance (Fig. 4). Similarly, differences in median MF diameter, myelin area, and axon area below as compared to above the cuff in controls and diabetics did not reach statistical significance.

**DISCUSSION**

In the present study we show that cuff compression of the mobilized peroneal nerve of rat at 150 mmHg for 30 min induces axonal degeneration of approximately one-third of MFs below the level of the cuff. In an earlier study, using the same cuff employed in study 1, we had found that nerve compression also induces demyelination and remyelination of a proportion of the MFs at the level of the cuff (11).

Among the times tested, 14 days appeared to be the most appropriate time because the pathologic abnormalities were better expressed than at 3 days or at 24 days. At 3 days the characteristic pathologic abnormalities of axonal degeneration in teased fibers were not yet fully expressed and by 21 days the characteristic changes had partially disappeared. At 3 days, myelin ovoids may have been included spuriously in morphometric results, thus overestimating the numbers of intact fibers. At 24 days, myelin ovoids and balls in degenerating fibers were small and widely spaced or nonexistent. At this time, regenerating fibers were well developed and appear as normal fibers. Our studies suggest that grading of teased fibers was more sensitive for detection of fiber degeneration than was estimating the numbers and sizes of MFs.
A statistically significant higher frequency of fiber abnormality distal to the cuff in controls than in rats with streptozotocin-induced diabetes was found at 3 and 14 days after cuff compression. The frequency of abnormality was also higher in controls than in diabetics at 24 days, but statistical significance was not attained possibly because myelin degenerative products had disappeared from some nerve strands and regenerating fibers had developed that could dilute the differential effect. The frequency of fiber regeneration was not evaluated because it would be expected to be decreased in the diabetics as a result of fewer fibers having degenerated.

Why was there a statistically significant difference of number of MFs per nerve in the distal as compared to proximal nerve not demonstrated in control as compared to diabetic nerve when a statistically significant increase in teased fibers undergoing degeneration was demonstrated? First, in teased fibers one estimates directly the rate of degenerating fibers, whereas in morphometric assessment nondegenerated and regenerating MFs are estimated—an indirect measure of fiber degeneration. Second, even by 14 days considerable axonal regeneration into the distal nerve segment is expected and was observed. The frequency of regeneration would be expected to be higher in nerves with higher frequency of degeneration, since regeneration follows degeneration, thus diminishing differences between controls and diabetics.

It has been stated that nerves in diabetics are more vulnerable to acute compression (1, 2), but there is evidence to the contrary. Paresthesia and increase of the vibratory detection threshold and development of nerve conduction block fail sooner during compression in controls than in diabetics (3–6). The present study extends previous studies to show that diabetes not only retards the development of functional alterations of nerve fibers but also inhibits degeneration of fibers.

How can resistance to compression axonal degeneration in experimental diabetes be explained? It seems unlikely that it could be due to alterations in the amount of endoneurial fluid or in its constituents because the endoneurial fluid is assumed to be displaced from beneath the cuff in controls and diabetics. The difference also cannot be explained by differences in numbers of fibers in control and in diabetic rats because these were not different. The fiber attenuation that occurs in untreated diabetes might predispose diabetics, and not controls, to fiber degeneration. The low endoneurial content of myo-inositol found in this study also might predispose diabetic nerves to fiber degeneration (12–14), but the converse was found arguing against a role of myo-inositol deficiency in predisposing nerve to fiber injury from acute compression. It may be that the bases for resistance to ischemic conduction block and for resistance to compression axonal degeneration are the same—a greater store of energy-producing substrate or a decrease in metabolic requirement of nerve fibers in diabetics (15, 16).

The present study may have implications for understanding nerve vulnerability to compression and entrapment. Gilliatt and Harrison (17) made a distinction between acute compression and entrapment nerve injuries, with increased pressure known to be involved in the former but not known to be involved in the latter. On the other hand, repeated minor degrees of compression of the nerve, particularly during unusual joint activity or position, may be involved along with other mechanisms in entrapment nerve injury. The relative contributions of mechanical displacement of axoplasm, ischemia, stretch, tethering, and other tissue alterations to nerve injury in acute compression and entrapment are still unresolved (18–23).

Assuming that compression neuropathy is more common in diabetics than in controls, mechanisms other than increased vulnerability of diabetic nerve fibers must be implicated. The present studies strongly imply that diabetic nerve fibers are less vulnerable to compression injury. Pathologic alterations of the connective sheaths of nerve or of the ligaments overlying nerves—e.g., alteration of the amount and flexibility of connective tissue as a consequence of protein glycation—might be a basis for increased compression of the median nerve by the carpal ligaments (24).