Impairment of Spinal Cord Conduction Velocity in Diabetic Rats

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Peripheral neuropathy is a common and well-studied complication of diabetes mellitus, but the possibility that central neuropathy is also present has received scant attention. Based on recent evidence showing that insulin has a direct effect on axon formation and neuronal survival in vitro, it was predicted that functional neuropathy would be present in the spinal cord of diabetic animals. Although structural lesions are encountered in the spinal cord of diabetic patients at autopsy, the functional corollaries have essentially remained unstudied. We used a new procedure to study evoked spinal cord potentials in the rat, which revealed a significant retardation in conduction velocity in streptozocin (STZ)-induced diabetic animals. This retardation was not due to a toxic effect of STZ on the involved spinal cord sensory pathways, because insulin infusion prevented the development of spinal cord neuropathy. The kinetics and magnitude of decline in conduction velocity were similar in the spinal cord, saphenous nerve, and common peroneal nerve during the first 2 wk, suggesting that a common mechanism was involved. After 10 wk, a spontaneous improvement in function was observed in the spinal cord and common peroneal nerve but not in the saphenous nerve. Our results support the hypothesis that central nervous system dysfunction can occur along with peripheral sensory neuropathy in diabetes.
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Diabetic neuropathy is an enigmatic disorder without consensus as to its pathology or pathogenesis (1,2). To date, most attention has been directed toward peripheral neuropathy. There are several informative reviews on this subject (2–5). In contrast, few research articles have been devoted to neuropathy in the central nervous system. Recent findings on the direct effect of insulin on neurons have led us to investigate the possibility of a diabetes-induced central neuropathy (6,7).

Emerging evidence suggests that insulin has direct effects on the nervous system. For example, physiological concentrations of insulin can enhance neurite (axon or dendrite) outgrowth and support the survival of sensory and sympathetic neurons (8). There is a close correlation between the concentrations of insulin that support neurite outgrowth (9), occupy the high-affinity insulin receptors (10), and increase the abundance of transcripts coding for structural proteins of neurites (11) in human neuroblastoma cells. Insulin shares important properties and a common mechanism with the classic neurotrophic polypeptide nerve growth factor (6,12). These observations are intriguing because the well-known syndrome of sensory and autonomic diabetic neuropathy includes loss of ganglion cells and decreased numbers and diameters of nerve fibers. Other neurophysiological effects of insulin include modulating the firing frequency of neurons (13), influencing catecholamine turnover (14), and promoting precocious maturation of synapses (15).

The observations suggest that a decline in insulin activity might produce neuropathy in other tissues containing insulin-responsive neurons, i.e., the central nervous system. Immunoreactive insulin and insulin receptors are widely present in the brain (16–19). Insulin supports neurite outgrowth and/or the survival of brain and spinal cord neurons (20–22).

A review of the literature reveals that spinal cord structural pathology is present in clinical and experimental diabetes. Autopsy shows degeneration in the spinal cord of diabetic patients (23), which can involve the spinocerebellar tracts (24,25) with loss of anterior horn cells (24,26,27). A reduction in perikaryal volume in ventral horn neurons, moreover, is observed in rats that have had diabetes for 4 wk (28). Although motor neuropathy is a prominent complication in diabetes (5,29), the case for spinal cord neuropathy has been difficult to make because *1*) all of its functional manifestations would tend to be masked in the usual tests by concomitant

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peripheral neuropathy, and 2) autopsy findings may have resulted from degenerative processes caused by age or may have occurred secondarily from debilitation in long-standing diabetes.

One of our aims was to further our understanding of the neurophysiological role of insulin. Streptozocin-induced diabetes (STZ-D) is an experimental insulin ablation condition that we used to test the hypothesis that insulin has neurotrophic properties (8). In particular, we wanted to ascertain whether there is functional impairment of the spinal cord in STZ-D rats secondary to insulin ablation. The second aim was to find out whether there is functional pathology in the diabetic spinal cord, regardless of cause. There is a need to describe more completely the nature and extent of central nervous system abnormalities in diabetes. We used a newly developed procedure to measure evoked spinal cord potentials in rats in this first demonstration of decreased spinal cord conduction velocity in experimentally induced diabetes.

MATERIALS AND METHODS

STZ, porcine insulin, and glucose diagnostic kit 510 were purchased from Sigma (St. Louis, MO). A miniosmotic pump was purchased from Alza (Palo Alto, CA; model 2ML1). Sprague-Dawley rats were obtained from Charles River (Wilmington, MA).

Rats were raised on standard rat chow and water ad libitum. At 12 wk of age, male rats were fasted overnight and anesthetized intramuscularly with 80 mg/kg ketamine and 0.3 mg/kg acepromazine. Diabetes mellitus was induced with 40 mg/kg i.v. STZ. Serum glucose concentrations were determined the following day with the glucose diagnostic kit. Only STZ-injected rats with >22 mM (400 mg/dl) glucose were included in the study. In addition, two groups of normal (untreated) control rats, 12 and 22 wk of age, were used.

At various times after the induction of diabetes, groups of five or six animals were fasted for 16 h and anesthetized intraperitoneally with a combination of 50 mg/kg ketamine and 20 mg/kg xylazine. Depth of anesthesia was monitored, and supplemental ketamine (25 mg/kg i.m.) was given as needed. The subcutaneous temperature at each recording site was monitored during the experiment with a needle thermistor (model 513; YSI, Yellow Springs, OH) and recording thermometer (model 43TF YSI). Body temperature was maintained within 1.5°C of normal with a circulating water blanket. A warmed mineral oil–petroleum jelly ointment was applied to exposed nerve segments to prevent dessication. The distance between stimulating and recording electrodes was monitored for nerve and spinal cord studies. After the electrophysiological measurements, blood was drawn for glucose determination by the glucose oxidase and peroxidase methods (30).

Saphenous nerve. A ground electrode was placed into the subcutaneous tissue near the medial aspect of the stifle. The nerve was gently dissected away from the adjacent artery and vein at proximal and distal sites. Stimulating silver chloride bipolar hook (SCBH) electrodes were placed 3 mm apart under the nerve at the proximal site near the femoral triangle, with the cathodal electrode distal to the anodal electrode. Recording SCBH electrodes were placed 3 mm apart under the nerve at the distal site near the ankle joint. The active recording electrode was placed closest to the cathodal stimulating electrode, and the nerve was severed distal to the reference electrode.

Common peroneal nerve. The sciatic nerve and its branches were exposed by retracting the biceps femoris and gluteal muscles. The semimembranosus-semitendinosus muscular branch was severed. Then the lateral cutaneous sural, caudal cutaneous sural, and tibial nerves were transected. Connective tissue was dissected away from the sciatic nerve proximal to the semimembranosus-semitendinosus muscular branch and from the common peroneal nerve near the stifle. Stimulating and recording SCBH electrodes (3-mm separation) were placed under the proximal and distal nerve sites, respectively. The electrode arrangement was similar to that for the saphenous nerve. The nerve was severed distal to the reference electrode, and a ground electrode was placed into the gluteal muscles.

Spinal cord. The following procedure was used to measure orthodromic spinal cord conductance. The 3rd cervical vertebra (C3) and the 8th–12th thoracic dorsal spinous processes (T8–12) were surgically exposed. Epaxial muscles were removed from the C2–3 interarcuate space, and the surgical fields were irrigated with 0.25 ml (2%) lidocaine.

The vertebral column was stabilized by securing the C2 dorsal spinous process with an Allis tissue forceps mounted to a micromanipulator. The previously exposed sciatic nerve was severed, and the proximal portion was placed across stimulating SCBH electrodes, with the cathode proximal to the anode. A ground was placed into the subcutaneous tissue in the caudal lumbar region. A Teflon-coated needle electrode (TECA, model MG25) with a 2-mm bared tip was placed into the epaxial muscles adjacent to the C2 dorsal lamina. A second recording electrode with a 1-mm bared tip was passed through the C2–3 interarcuate ligament into the epidural space overlying the dorsal funiculus of the spinal cord, ipsilateral to the stimulated sciatic nerve. Evoked spinal cord potentials were recorded at C2–3 after stimulation of the sciatic nerve.

Next, the dorsal spinous processes of T10–11 were clamped, and the process at T9 was removed. The electrode with the 2-mm bared tip was placed into the adjacent left epaxial muscles. An electrode with a 1-mm bared tip was passed through the T8–9 interarcuate ligament into the epidural space overlying the ipsilateral dorsal funiculus, and evoked spinal cord potentials were recorded as before.

Stimulus. Square-wave pulses of 25 μs duration, at a rate of 3–5/s, were used to evoke compound action potentials in the saphenous and common peroneal nerves. The threshold stimulus to generate an action potential was below the limits for accurate determination by our instruments. Therefore, a 30-μA constant-current stimulus was selected because it provided maximum peak amplitude and stable configuration of the evoked compound action potentials.

Square-wave pulses (0.3–2.0 mA) of 50 μs duration, at a rate of 8–9/s, were used to evoke spinal cord potentials.

Recording. The amplified evoked potentials and synchronization pulses from the stimulator were put into a digital oscilloscope for signal averaging, then stored in a computer for analysis.

In the peripheral nerve, 32 successive responses to nerve stimulation were averaged for analysis of evoked potentials. Each averaged action potential contained 1024 data points,

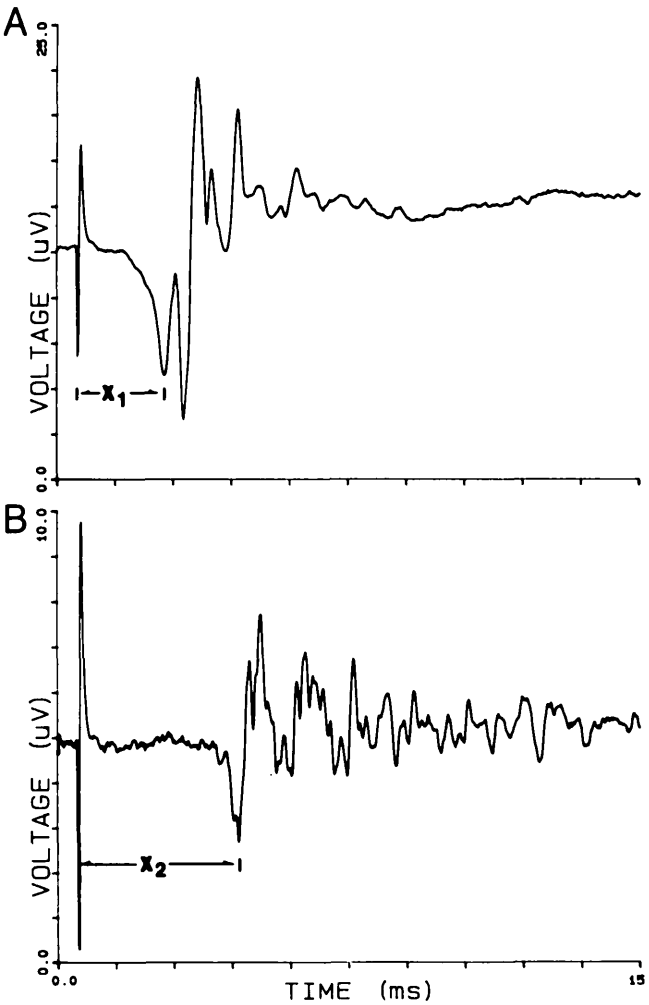

FIG. 1. Measurement of orthodromic spinal cord evoked potentials in diabetic rat. Diabetes was induced in 12-wk-old male rat. Evoked potentials are shown 6 wk after induction with stimulus electrode at sciatic nerve and recording electrodes at spinal cord levels T8–9 (*A*) and C2–3 (*B*). X_1 and X_2 are latencies measured from stimulation artifact to onset of 1st negative peak recorded at T8–9 and C2–3, respectively. Conduction velocity within spinal cord is calculated as measured distance between recording electrodes divided by difference in latencies ($X_2 - X_1$). Note difference in voltage scales between *A* and *B*.

with a sampling rate of 5 μs/point. The band pass was 3–10 kHz (−3 dB).

Each evoked spinal cord potential was the average of 1024 successive spinal cord responses to sciatic nerve stimulation, containing 1024 data points, with a sampling rate of 15 μs/point. The band pass was 3–30 kHz (−3 dB).

Analysis of evoked potentials. Peripheral nerve latencies were measured from the onset of the stimulus artifact to the onset of the monophasic negative (upward) compound action potential. Conduction velocities were calculated by dividing the distance between the stimulating and recording electrodes by the latency. The duration of the evoked potential was measured from the onset of the potential to its return to baseline. The method for calculating spinal cord conduction velocities is described below.

Insulin infusion and antidromic spinal cord conductance. Miniosmotic pumps were implanted subcutaneously in some groups immediately after STZ was administered. The conditions were similar to those described to maintain normoglycemia by constant infusion of insulin (31). The pumps were filled with either vehicle (1.6% glycerin, 7 mg/ml glutamic acid, 0.2% phenol, pH 3.3) or a vehicle containing porcine insulin that had been sterilized by passage through 0.2-μm filters. Insulin concentrations added to pumps were adjusted to deliver 10 U · kg^{-1} · day^{-1} at a rate of 240 μl/day. The filled pumps were soaked in saline for 2 h before implantation to decrease the lag in release of solution. Animals implanted with miniosmotic pumps and untreated control rats were not fasted before serum glucose assay because of the possibility of severe hypoglycemia in the insulin-treated group.

Antidromic conductance was studied in the spinal cords of untreated and pump-implanted rats 1 wk after STZ administration by investigators who had no knowledge of the treatment given to individual animals. The maximum spinal cord conduction velocities determined by both antidromic and orthodromic procedures are the same, because in each case the velocity arises in the most rapidly conducting primary afferent fibers (R.E.C., L.R.W., and T.M. Brushart, unpublished observations). The anesthetic, surgical, and electrophysiological methods were the same as those described above, except for the arrangement of stimulating and recording electrodes. The sciatic nerve was severed, and the proximal portion was placed across SCBH electrodes. Teflon-coated needle electrodes with 1-mm bared tips were passed through the C2–3 interarcuate ligament into the epidural space and served as stimulating electrodes. The rats were given pancuronium bromide (0.6 mg/kg) and were artificially ventilated. Compound action potentials recorded from the sciatic nerve were produced by averaging 64 responses to spinal cord stimulation. After stimulation at C2–3, stimulating electrodes were placed through the T8–9 interarcuate space. Evoked potentials were next recorded from the sciatic nerve after stimulation at T8–9. The latency of averaged evoked potentials was measured from the stimulus artifact to the onset of the monophasic potential, the distance between the C2–3 and T8–9 sites was measured, and the conduction velocity was calculated.

The results are calculated as means ± SE for groups of five or six animals. Statistical calculations were made with Student's *t* test.

RESULTS

Measurement of evoked spinal cord potentials in rats. An extensive literature search did not uncover a published procedure for the measurement of the conduction velocity of evoked spinal cord potentials in the rat; therefore, procedures used in other animals (32) were modified for use in the rat (R.E.C., L.R.W., and T.M. Brushart, unpublished observations). The sciatic nerve was stimulated, and recordings were taken at T8–9 (Fig. 1*A*) and at C2–3 (Fig. 1*B*), a site farther from the stimulus. The most rapidly conducting fibers gave rise to the earliest peaks in the compound action potentials. For the most rapidly conducting fibers, the interval between the stimulation artifact and the onset of the first negative peak represents the conduction time between stimulation and recording sites; the interval is shown as X_1 and X_2 in Fig. 1. The latency was, of course, longer when recorded at the C2–3 site. By determining the difference in

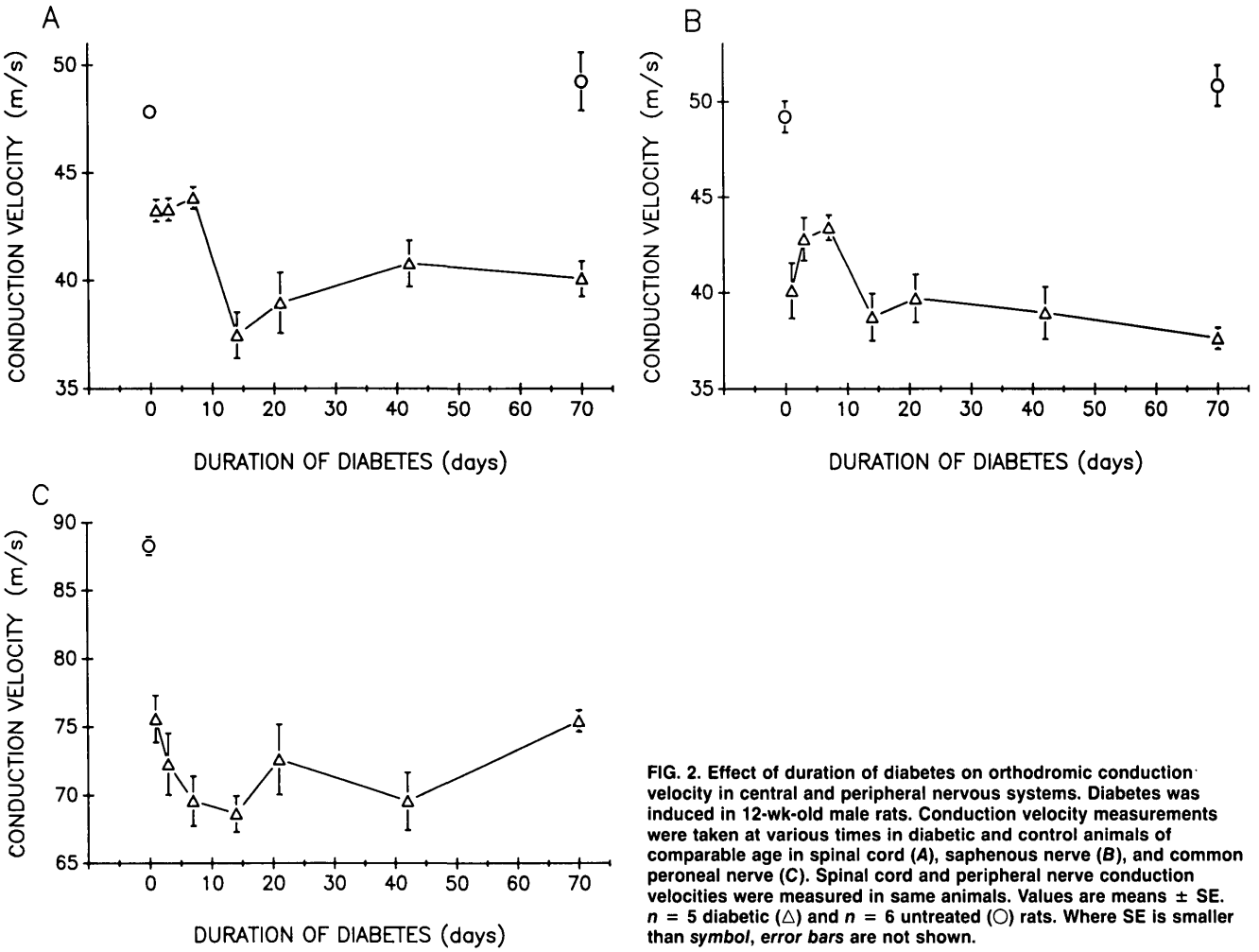

FIG. 2. Effect of duration of diabetes on orthodromic conduction velocity in central and peripheral nervous systems. Diabetes was induced in 12-wk-old male rats. Conduction velocity measurements were taken at various times in diabetic and control animals of comparable age in spinal cord (*A*), saphenous nerve (*B*), and common peroneal nerve (*C*). Spinal cord and peripheral nerve conduction velocities were measured in same animals. Values are means ± SE. *n* = 5 diabetic (△) and *n* = 6 untreated (○) rats. Where SE is smaller than *symbol*, *error bars* are not shown.

latency ($X_2 - X_1$) of evoked potentials recorded at the two levels of the spinal cord and measuring the distance between the two recording electrodes, the maximum conduction velocity within the spinal cord was calculated. A more complete description of the procedure is available (R.E.C., L.R.W., and T.M. Brushart, unpublished observations). The conduction velocity was essentially the same in normal male rats between 12 (47.3 ± 1.1 m/s, n = 5) and 22 (49.3 ± 2.7 m/s, n = 5) wk of age, which was the age range of animals used in this study.

Effect of duration of diabetes on conduction velocity in spinal cord, saphenous nerve, and common peroneal nerve. The kinetics of decline in spinal cord conduction velocity appeared to be biphasic. In the initial phase, there was a significant rapid decline in spinal cord conduction velocity within the 1st day after induction of diabetes (Fig. 2*A*). Between 1 and 7 days, there was no significant change. This was followed by a second phase of significant decline ($P < .001$), which occurred between 1 and 2 wk of diabetes. The maximum reduction in conduction velocity was observed at ~2 wk. There was a significant improvement ($P < .05$) between 2 and 6 wk of diabetes, which was sustained at 10 wk. Nevertheless, conduction velocity was still significantly decreased ($P < .005$) in rats with chemically induced diabetes at 10 wk (22 wk old) compared with 22-wk-old untreated control animals.

The diabetic rat had reduced amplitudes of spinal cord action potentials as well as decreased conduction velocity. This was consistently observed in many animals, not only for the most rapidly but also for the more slowly conducting peaks. The reduced amplitudes may indicate a diminution in the number of functionally conducting fibers.

The effect of duration of diabetes was studied on the velocities of the most rapidly conducting fibers in the saphenous (Fig. 2*B*) and common peroneal (Fig. 2*C*) nerves to describe in detail the temporal changes in nerve function after the induction of this disorder, particularly early after onset. In both the common peroneal and saphenous nerves, conduction velocity was significantly reduced ($P < .001$) 1 day after the induction. The maximum decrease was reached in ~2 wk, and the conduction velocities of both nerves remained significantly reduced after 10 wk of diabetes.

Comparison of effect of duration of diabetes on relative magnitude of change in conduction velocity in spinal cord and peripheral nerves. The data from Fig. 2 were replotted as percentages relative to the values for untreated 12-wk-old animals. The results are shown in Fig. 3. There were similarities in the time course and magnitude of decline in relative conduction velocities of action potentials among the three tissues. The biphasic decline in conduction velocity was present in both the saphenous nerve and the spinal cord. In contrast, the common peroneal nerve showed a

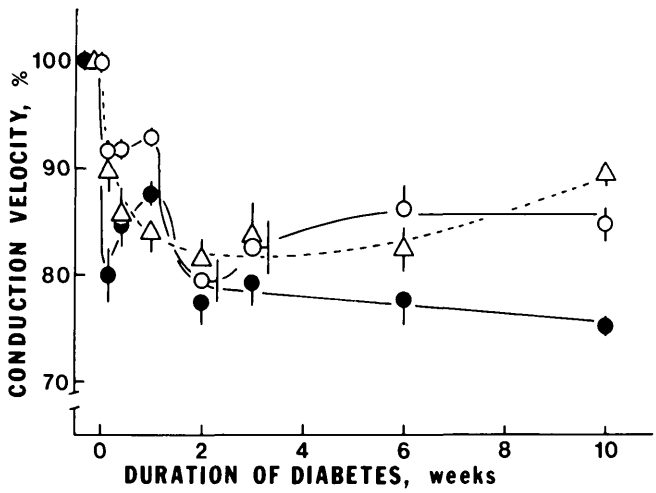

FIG. 3. Comparison of relative magnitude of change in conduction velocities in spinal cord (○), saphenous nerve (●), and common peroneal nerve (△) after diabetes induction. Data in Fig. 2 were replotted in percentages relative to values in untreated 12-wk-old rats.

progressive decline in conduction velocity over 2 wk; the half time for this decline was ~1 day. The maximum decrease in conduction velocity occurred within 2 wk in all three tissues. Moreover, the maximum decrease in conduction velocities was comparable among the three tissues and approached 20% of the values in untreated controls. These results suggest that a common mechanism underlies the decrease in conduction velocities.

Spontaneous recovery in conduction velocity. Conduction velocities were significantly recovered in the common peroneal nerve ($P < .005$) and spinal cord ($P < .05$) between 2 and 10 wk after diabetes induction. The magnitude of the spontaneous recovery in both cases was about the same. In contrast, there was no improvement in the saphenous nerve.

The fasting blood glucose levels were essentially the same among the 2- and 10-wk-diabetic animals (2 wk 39.5 ± 7.7 mM, 10-wk 33.7 ± 2.3 mM; means ± SE, $n = 5$), indicating that improvement in conduction velocity was not due to a decrease in blood glucose levels toward normal. The mean fasting serum glucose concentration at the time of conduction velocity determinations for all diabetic animals in this study was 35.5 ± 1.7 mM ($n = 34$). In addition, the mean body weight of 10-wk-diabetic rats (273 ± 15 g) was significantly lower ($P < .01$) than that of 2-wk-diabetic rats (330 ± 12 g). These data indicate that the overall condition of the rats continued to decline, although there was an improvement in conduction velocity in the spinal cord and common peroneal nerve.

Effect of insulin infusion on development of spinal cord neuropathy. STZ might be toxic to cells in the spinal cord, leading to reduced conduction velocity. To study this possibility, miniosmotic pumps were implanted at a subcutaneous site at the time of STZ administration. As expected, spinal cord conduction velocity was reduced in animals that received vehicle only. The decline in conduction velocity was, however, prevented in animals that received insulin (Fig. 4). Hyperglycemia did not develop in the insulin-treated group.

Antidromic, rather than orthodromic, conductance was studied in this experiment. The velocities of the most rapidly conducting fibers were the same for antidromic (Fig. 4) and orthodromic (Fig. 2*A*) conductances in untreated animals. In addition, the magnitude of decline in conduction velocity was very similar under both conditions of measurement in animals that were diabetic for 1 wk.

DISCUSSION

The measurement of conduction velocities of evoked spinal cord potentials provides a useful method for studying functional neuropathy in the central nervous system of the rat. To our knowledge, these results are the first to demonstrate that there is a significant decline in spinal cord conduction velocity of compound action potentials in experimental diabetes. Futhermore, the results show that peripheral sensory neuropathy and spinal cord dysfunction can present with a similar time course in experimental diabetes.

Action potentials of primary afferent fibers are responsible for the onset of evoked spinal cord potentials recorded at the thoracic and cervical sites (R.E.C., L.R.W., and T.M. Brushart, unpublished observations). Therefore, the maximum conduction velocity of evoked spinal cord potentials reflects activity in these fibers, which are found in a well-delineated region of the fasciculus gracilis. The same maximum velocities were observed when antidromic conduction was studied (Figs. 2*A* and 4). The reduction in maximum orthodromic and antidromic conduction velocities for evoked spinal cord potentials suggests that STZ-D results in functional alterations in these fibers. However, this impairment may not be restricted to the largest-diameter primary afferent fibers of dorsal root ganglion cells. Because degeneration of the spinocerebellar tracts is observed in protracted clinical diabetes (23–25), the function of fibers that are part of other ascending systems, the cell bodies and axons of which are found entirely within the central nervous system, might also be affected. Other tests are being developed to delineate more clearly the ascending sensory and descending motor fiber tracts that give rise to particular peaks comprising the

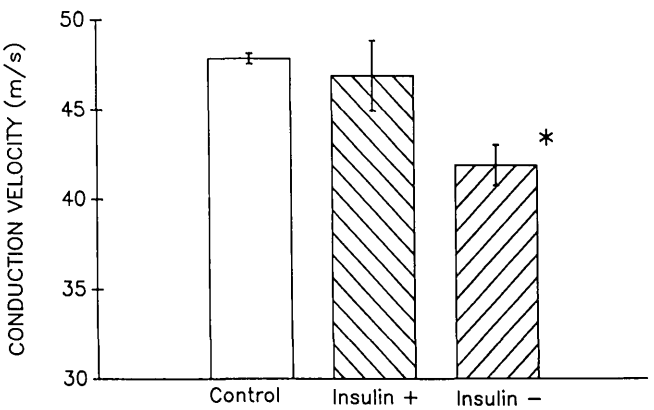

FIG. 4. Effect of insulin infusion on reduction of antidromic spinal cord conduction velocity induced by streptozocin (STZ)-induced diabetes. Twelve-wk-old rats were randomly assigned to 3 treatment groups, 1 of which received no treatment (*open bar*). STZ was administered to 2 groups, and miniosmotic pumps were implanted subcutaneously. Pumps delivered either 10 U of insulin · kg^{-1} · day^{-1} (insulin+) or vehicle (insulin−). One week after induction of diabetes, antidromic spinal cord conduction velocity was measured. Vehicle group was significantly different from control and insulin+ groups. *$P < .05$.

evoked spinal cord action potentials and to identify which tracts are affected in diabetes.

The rapid decline in conduction velocity, which is evident within 1 day of induction of diabetes in the spinal cord, common peroneal nerve, and saphenous nerve, is also seen in the tail nerve (33). Consistent with these results, a reduction in the perikaryal volume of the ventral horn motor neurons is observed in rats that were diabetic for 4 wk (28).

The relationship between duration of diabetes and alteration in conduction velocity was studied in central and peripheral fibers. Comparable clinical data of this type would be difficult to come by. These results may be useful for future studies aimed at correlating biochemical, ultrastructural, and functional derangements in experimental diabetes.

The unexpectedly complex rate of onset, partial recovery, and tissue-dependent nature of the kinetics of functional neuropathy are observations that must be considered in any theory of pathogenesis. It is curious that the spinal cord and the saphenous nerve appear to rally between 1 and 7 days after diabetes induction. Eventually there was a spontaneous partial recovery of conduction velocity in the common peroneal nerve and the spinal cord between 2 and 10 wk after diabetes induction (Figs. 2 and 3). Regarding neuropathy, partial recovery is often observed clinically (5). There is a negative correlation between elevated plasma glucose concentrations and conduction velocity in diabetic patients (34). Thus, the partial recovery might have been due to a spontaneous reduction in hyperglycemia. However, blood samples taken on the same day as conduction velocity measurements showed that the recovery was not due to a corresponding improvement (lowering) in glucose concentrations. In the common peroneal nerve, partial recovery might be attributed to delayed maturation of myelinated fibers (35). However, in the spinal cord, further developmental increases in the maximum spinal cord conduction velocity have not been observed after 12 wk of age, and spontaneous partial recovery in diabetic rats is not attributed to delayed maturation. Because there was no concurrent recovery in the saphenous nerve in the same animals, the common peroneal nerve and spinal cord appear to have an intrinsic advantage in their capacity to support recovery. Although both the saphenous and peroneal nerves contain cutaneous afferent fibers, only the peroneal nerve has a large component of muscle afferent and motor fibers. Whatever the basis for recovery, these results suggest that after an acute decline in insulin activity, the magnitude of the conduction velocity deficit detected may depend on the time elapsed after the decline, as well as on the particular nerve selected for examination.

STZ-D is an insulin ablation experiment. The results, which support our prediction that spinal cord functional neuropathy does occur in diabetes, may be interpreted as additional, albeit indirect, support for the hypothesis that insulin may help regulate conduction velocity in neurons. Several lines of evidence support this hypothesis. For example, physiological concentrations of insulin can directly enhance axon formation in sensory, sympathetic, and spinal cord neurons (8,22). Insulin can modulate the abundance of neurofilament (36) and tubulin (11) mRNAs in a manner closely correlated with its capacity to induce neurite elongation. Conduction velocity is dependent, in part, on the diameter of axons. Evidence suggests that the diameter is particularly dependent on neurofilament proteins and gene expression (37). Microtubules, comprised of α- and β-tubulin heterodimers, are major constituents of the axonal cytoskeleton and also support axonal transport. Decreased insulin activity may result in diminished production of axonal cytoskeletal proteins, reduced axonal transport, loss of axons, and loss of neurons. These events, together with the metabolic alterations described by others, might explain, in part, the decreased conduction velocity and amplitude of compound action potentials.

The basis for the biphasic decline in conduction velocity in the saphenous but not common peroneal nerve is not understood. However, various populations of neurons do display differential sensitivity to insulin (8). Furthermore, insulinlike growth factors and insulin share overlapping effects and a common mechanism in neurons (6,7). The second phase in the decline of conduction velocity follows remarkably closely the kinetics of decline in insulinlike growth factor I mRNA levels after the induction of diabetes (unpublished observations). These observations suggest that the reduced activity of insulin and other neurotrophic factors may contribute to neuropathy in diabetes.

Whatever the cause, these results add to the small but growing number of observations indicating that there can be both structural and functional pathology in the central nervous system in diabetes. Functional disturbances are seen in the brain, where a delay in auditory brain stem responses is reported (38). Moreover, degeneration is observed in the cells and nerve fibers of the brain stem, cerebrum, and cerebellum (27,39).

The possibility that STZ toxicity may have caused the change in spinal cord conduction velocity is remote for a number of reasons. Insulin infusion prevented the development of spinal cord neuropathy (Fig. 4). This finding showed that neuropathy was not directly caused by STZ toxicity to the primary afferent pathway. In a related observation, STZ-induced peripheral nerve neuropathy is reversible when animals are quickly treated with insulin (33,40). Finally, during the course of writing this manuscript, we learned that Pozzessere et al. (41) published a report showing that electrophysiological abnormalities are present in the central nervous system (visual and brain stem auditory evoked potentials) in both insulin-dependent and non-insulin-dependent diabetic patients with disease duration <4 yr. Therefore, it appears that the STZ-D rat may model an early central neuropathy that is clinically manifested. It is also important that the predictions of the model are borne out in both the clinical and experimental setting.

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REFERENCES

1. Powell HC: Pathology of diabetic neuropathy: new observations, new hypotheses. *Lab Invest* 49:515–18, 1982
2. Thomas PK, Eliasson SG: Diabetic neuropathy. In *Peripheral Neuropathy*. Vol. 2. Dyck PJ, Thomas PK, Lambert EH, Bunge R, Eds. Philadelphia,

PA, Saunders, 1984, p. 1773–810
3. Clarke BF, Ewing DJ, Campbell IW: Diabetic autonomic neuropathy. *Diabetologia* 17:195–212, 1979
4. Hilsted J: Pathophysiology in diabetic autonomic neuropathy: cardiovascular, hormonal and metabolic studies. *NY State J Med* 82:892–903, 1982
5. Dyck PJ, Thomas PK, Asbury AK, Winegrad AI, Porte D Jr: *Diabetic Neuropathy*. Philadelphia, PA, Saunders, 1987
6. Ishii DN, Mill JF: Molecular mechanisms of neurite formation stimulated by insulin-like factors and nerve growth factor. *Curr Top Membr Transp* 31:31–78, 1987
7. Recio-Pinto E, Ishii DN: Insulin and related growth factors: effects on the nervous system and mechanism for neurite growth and regeneration. *Neurochem Int* 12:397–414, 1988
8. Recio-Pinto E, Rechler MM, Ishii DN: Effects of insulin, insulin-like growth factor-II, and nerve growth factor on neurite formation and survival in cultured sympathetic and sensory neurons. *J Neurosci* 6:1211–19, 1986
9. Recio-Pinto E, Ishii DN: Effects of insulin, insulin-like growth factor-II and nerve growth factor on neurite outgrowth in cultured human neuroblastoma cells. *Brain Res* 302:323–34, 1984
10. Recio-Pinto E, Ishii DN: Insulin and insulinlike growth factor receptors regulating neurite formation in cultured human neuroblastoma cells. *J Neurosci Res* 19:312–20, 1988
11. Mill JF, Chao MV, Ishii DN: Insulin, insulin-like growth factor II, and nerve growth factor effects on tubulin mRNA levels and neurite formation. *Proc Natl Acad Sci USA* 82:7126–30, 1985
12. Fernyhough P, Ishii DN: Nerve growth factor modulates tubulin transcript levels in pheochromocytoma PC12 cells. *Neurochem Res* 12:891–99, 1987
13. Oomura Y, Kita H: Insulin acting as a modulator of feeding through the hypothalamus. *Diabetologia* 20:290–98, 1981
14. Sauter A, Goldstein M, Engel J, Ueta K: Effect of insulin on central catecholamines. *Brain Res* 260:330–33, 1983
15. Puro DG, Agardh E: Insulin-mediated regulation of neuronal maturation. *Science* 225:1170–72, 1984
16. Baskin DG, Porte D Jr, Guest K, Dorsa, DM: Regional concentrations of insulin in the rat brain. *Endocrinology* 112:898–903, 1983
17. Le Roith D, Hendricks SA, Lesniak MA, Rishi S, Becker KL, Havrankova J, Rosenzweig JL, Brownstein MJ, Roth J: Insulin in brain and other extrapancreatic tissues of vertebrates and nonvertebrates. *Adv Metab Disord* 10:303–40, 1983
18. Posner BI, Kelly PA, Shiu RPC, Friesen HG: Studies of insulin, growth hormone and prolactin binding: tissue distribution, species variation and characterization. *Endocrinology* 95:521–31, 1974
19. Havrankova J, Roth J, Brownstein M: Insulin receptors are widely distributed in the central nervous system of the rat. *Nature* (Lond) 272:827–29, 1978
20. Bhat NR: Insulin dependent neurite outgrowth in cultured embryonic mouse brain cells. *Dev Brain Res* 11:315–18, 1983
21. Aizenman Y, Weichsel ME Jr, de Vellis J: Changes in insulin and transferrin requirements of pure brain neuronal cultures during embryonic development. *Proc Natl Acad Sci USA* 83:2263–66, 1986
22. Glazner G, Ishii DN: Insulin, insulinlike growth factor-I, and nerve growth factor stimulate neurite formation in spinal cord cultures. *Soc Neurosci Abstr* 14:1040, 1988
23. Woltman HW, Wilder RM: Diabetes mellitus: pathologic changes in spinal cord and peripheral nerves. *Arch Intern Med* 44:576–603, 1929
24. Olsson Y, Save-Soderbergh J, Sourander P, Angervall LA: Patho-anatomical study of the central and peripheral nervous system in diabetes of early onset and long duration. *Pathol Eur* 3:62–79, 1968
25. Slager UT, Webb AT: Pathologic findings in the spinal cord. *Arch Pathol* 96:388–94, 1973
26. Greenbaum D, Richardson PC, Salmon MV, Urich H: Pathological observations on six cases of diabetic neuropathy. *Brain* 87:201–14, 1964
27. Reske-Nielsen E, Lundbaek K: Pathological changes in the central and peripheral nervous system of young long-term diabetics. II. The spinal cord and peripheral nerves. *Diabetologia* 4:34–43, 1968
28. Sidenius P, Jakobsen J: Reduced perikaryal volume of lower motor and primary sensory neurons in early experimental diabetes. *Diabetes* 29:182–87, 1980
29. Gregersen G: Diabetic neuropathy: influence of age, sex, metabolic control, and duration of diabetes on motor conduction velocity. *Neurology* 17:972–80, 1967
30. Raabo E, Terkildsen TC: On the enzymatic determination of blood glucose. *Scand J Clin Lab Invest* 12:402–407, 1960
31. Patel DG: Rate of insulin infusion with a minipump required to maintain a normoglycemia in diabetic rats (41529). *Proc Soc Exp Biol Med* 172:74–78, 1983
32. Holliday TA, Weldon NE, Ealand BG: Percutaneous recording of evoked spinal cord potentials of dogs. *Am J Vet Res* 40:326–33, 1979
33. Jakobsen J: Early and preventable changes of peripheral nerve structure and function in insulin-deficient diabetic rats. *J Neurol Neurosurg Psychiatry* 42:509–18, 1979
34. Graf RJ, Halter JB, Halar E, Porte D Jr: Nerve conduction abnormalities in untreated maturity onset diabetes. *Ann Intern Med* 90:298–303, 1979
35. Moore SA, Peterson RG, Felten DL, O'Connor BL: A quantitative comparison of motor and sensory conduction velocities in short- and long-term streptozotocin- and alloxan-diabetic rats. *J Neurol Sci* 48:133–52, 1980
36. Wang C, Wible B, Angelides K, Ishii DN: Insulin and insulinlike growth factor-I increase neurofilament mRNA and neurite formation. *Soc Neurosci Abstr* 14:1169, 1988
37. Hoffman PN, Cleveland DW, Griffin JW, Landes PW, Cowan NJ, Price DL: Neurofilament gene expression: a major determinant of axonal caliber. *Proc Natl Acad Sci USA* 84:3472–76, 1987
38. Donald MW, Bird CE, Lawson JS, Letemendia FJJ, Monga TN, Surridge DHC, Varette-Cerre P, Williams DL, Williams DML, Wilson DL: Delayed auditory brainstem responses in diabetes mellitus. *J Neurol Neurosurg Psychiatry* 44:641–44, 1981
39. Reske-Nielsen E, Lundbaek K, Rafaelsen OU: Pathological changes in the central and peripheral nervous systems of young long-term diabetics. I. Diabetic encephalopathy. *Diabetologia* 1:233–41, 1965
40. Greene DA, DeJesus PV, Winegrad AI: Effects of insulin and dietary myoinositol on impaired peripheral motor nerve conduction velocity in acute streptozotocin diabetes. *J Clin Invest* 55:1326–36, 1975
41. Pozzessere G, Rizzo PA, Valle E, Mollica MA, Meccia A, Morano S, Di Mario U, Andreani D, Morocutti C: Early detection of neurological involvement in IDDM and NIDDM: multimodal evoked potentials versus metabolic control. *Diabetes Care* 11:473–80, 1988