Rapid Publication

Spontaneous Activity of Primary Afferent Neurons in Diabetic BB/Wistar Rats

A Possible Mechanism of Chronic Diabetic Neuropathic Pain

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SUMMARY

The mechanism of painful diabetic neuropathy remains unknown. Spontaneous activity in nociceptive primary afferents has been implicated in the genesis of chronic pain due to peripheral nerve injury, and diabetic axonopathy shares some histologic features with traumatic neuropathy. We hypothesized that spontaneous hyperactivity of nociceptive neurons might represent the neurophysiologic mechanism of diabetic neuropathic pain. To test this, we examined the spontaneous activity of primary afferent axons from diabetic BB/Wistar and normal Wistar rat saphenous nerves isolated from central and peripheral connections. Microfilament recordings from diabetic nerves showed a significantly higher incidence of spontaneous discharges in comparison to normal nerves. Furthermore, this spontaneous hyperactivity occurred almost exclusively in potentially nociceptive C-fibers. We conclude that in the diabetic BB/Wistar rat, spontaneous impulses are generated in potential nociceptive primary afferent neurons, and that this may represent the mechanism of chronic diabetic neuropathic pain. DIABETES 1985; 34:1210–13.

Peripheral neuropathy is a common complication of diabetes mellitus, affecting more than one million individuals in the United States alone.[1] A common symptom of this disorder is neuropathic pain. Although the histologic, ultrastructural, and neurophysiologic sequelae of diabetic neuropathy have been well studied, the mechanism of diabetic neuropathic pain remains unknown. In most cases, diabetic polyneuropathy involves a combination of sensory, motor, and autonomic nerve fiber abnormalities. However, in some patients small-fiber pathology predominates, and pain and temperature sensibilities are disturbed. Disabling spontaneous pains, dysesthesias, and paresthesias are common in these individuals. The pains can be multifocal, shooting, or stabbing, but are commonly described as "burning" or "aching" pains that can be superficial or bone-deep.[2]

Diabetic polyneuropathy is best classified as an axonal neuropathy, in that the predominant neuropathic feature is nerve fiber loss.[2–5] In nerves of some diabetic patients, segmental demyelination and remyelination appear to occur independent of axonal loss.[2,3,6,7] In a morphometric study of two cases involving painful neuropathy, findings of increased numbers of small, unmyelinated axons suggested axonal regeneration with sprouting in concert with reduction of unmyelinated fibers of normal caliber.[2] These changes probably reflect concurrent fiber degeneration and regeneration, a pattern that also occurs in other axonal polyneuropathies.

The paradoxical coexistence of spontaneous pain and insensitivity to painful stimuli so commonly seen in diabetic individuals remains unexplained. However, it has been suggested that pain results from hyperactivity of injured small-diameter fibers.[2,8] Ochoa and Torebjork[9] have made microelectrode recordings within myelinated fibers of human peripheral nerve following periods of limb ischemia. These microneurographic recordings showed spontaneous multiunit neural activity in myelinated fibers that temporally correlated with paresthesias perceived by the subjects. Although unmyelinated fibers were apparently not involved in the spontaneous discharge, this work has often been cited as an indication of the subjective concomitants of spontaneous neural activity.

Wall et al. have demonstrated that regenerating fibers within neuromas of rats and mice manifest spontaneous burst discharges, mechanosensitivity, and chemosensitivity to alpha-adrenergic agonists.[10–13] Spontaneous discharges were found in small myelinated and unmyelinated fibers. Thus, spontaneous activity in a nociceptive axon with an injured, nonconducting distal segment would explain the finding of pain in an anesthetic region.

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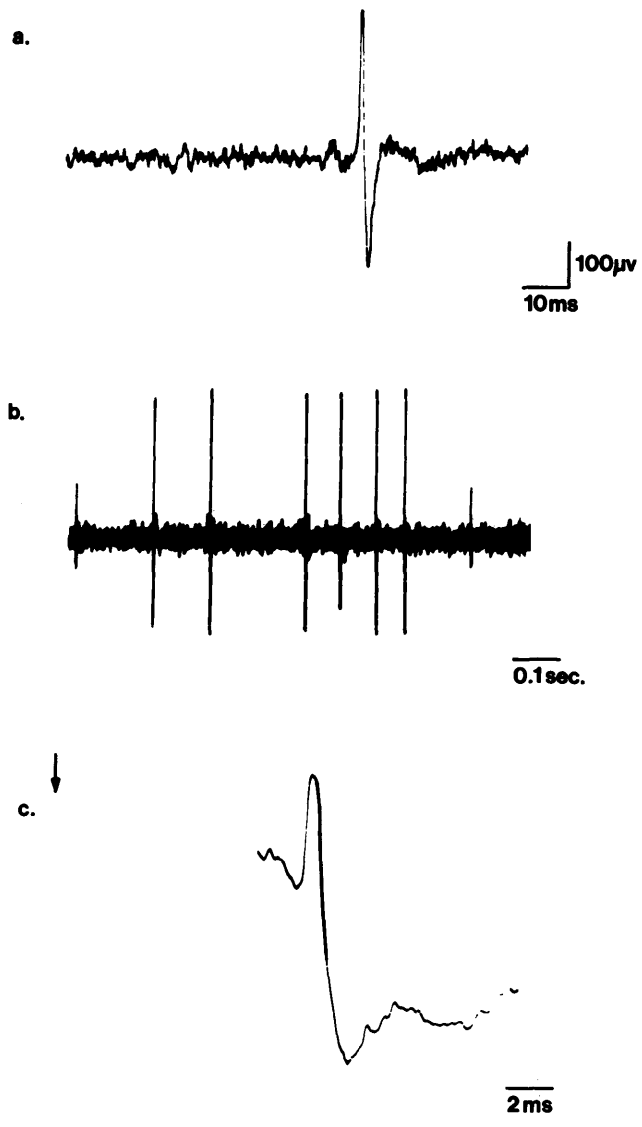

FIGURE 1. Spontaneous activity in an isolated segment of saphenous nerve from a diabetic BB/Wistar rat. (a and b) Action potential and spontaneous firing pattern of the isolated axon. (c) The axon responded to antidromic stimulation (arrow) with a latency of 12 ms (conduction distance = 20 mm), yielding a conduction velocity of 1.7 m/s, i.e., a C-fiber. The large A-fiber compound action potential has been subtracted for clarity.

Previous studies have described spontaneous discharges that originate in the dorsal root ganglion cell somata of myelinated and unmyelinated afferents of damaged peripheral nerves, and have implicated this abnormal neural activity in the genesis of traumatic neuropathic pain.[11,14-18] Spontaneous dorsal root ganglion discharge may, in fact, account for prolonged intractable pains and paresthesias that may follow nerve damage, including so-called "phantom" pains. Ectopic activity in the dorsal root ganglion may also in part explain the persistence of ongoing neural activity recorded (by the microneurographic technique) from transsected nerves in amputees after local anesthesia of the end-bulb neuroma.[19] The hypothesis of this study was that similar spontaneous neural activity occurs in diabetic neuropathy and is responsible for the pain and dysesthesias that often accompany this disorder.

The spontaneously diabetic BB rat was used as an animal model, since it consistently demonstrates functional and structural peripheral nerve abnormalities similar to those seen in human diabetic neuropathy.[20-25]

MATERIALS AND METHODS

Prediabetic male and female BB/Wistar rats were obtained from Health and Welfare Canada (courtesy of Dr. P. Thibert). Age-matched nondiabetic male and female Wistar rats served as controls. All animals were maintained in individual air-filtered metabolic cages with ad libitum access to rat chow (Wayne Lab Blosx F-6, Wayne Laboratory Animal Diets, Wayne Feed Division, Winnipeg, Manitoba, Canada) and water. Body weight, urine volume, and glucosuria (defined qualitatively by a positive Tes-Tape reaction (Eli Lilly Canada Inc., Toronto, Ontario, Canada) were monitored daily. Protamine zinc insulin (PZI, Connaught Laboratories Ltd., Toronto, Ontario), 1–3 U/day, was begun immediately following the detection of glucosuria to ensure survival. Recordings were obtained from 11 diabetic rats with diabetic duration ranging from 3.25 to 9 mo (4.9 ± 1.8 mo), and with a mean urine glucose value of 0.9 ± 0.07 g%.

Recordings from these animals were compared with 10 normal age-matched Wistar rats with urine glucose values undetectable with Tes-Tape. All animals were generally anesthetized with intraperitoneal (i.p.) pentobarbital (50 mg/kg), and a venous cannula was inserted for infusion of intravenous fluids and drugs when necessary. Respiration was controlled via tracheostomy for an end-expiratory CO_2 of 3.5%, and body temperature was kept at 38 ± 0.5°C. The temperature of the oil pool covering exposed neural structures was maintained at 37 ± 1°C with radiant heat.

A laminectomy at L2-3 was then performed and the cauda equina was divided at this level. Animals were then placed in the supine position, and the saphenous nerve was exposed in the thigh. The nerve was then dissected out as far proximally as possible and placed on bipolar silver stimulating electrodes. Distal dissection of the nerve was limited by branching of the nerve. Therefore, care was taken to ensure the entire nerve trunk was isolated as distally as possible. Under magnification, the nerve was then split into filaments using no. 5 jeweler's forceps. Filaments were selected that

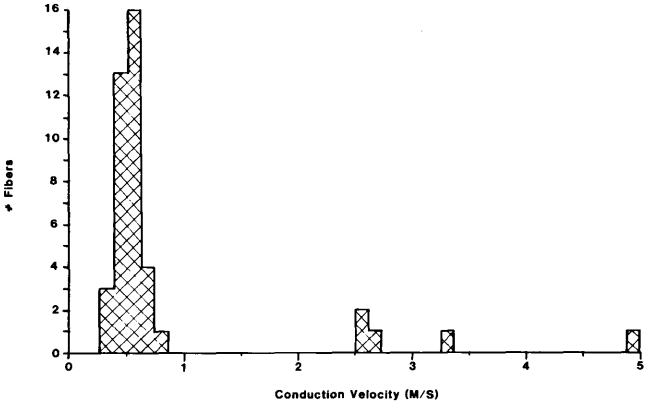

FIGURE 2. Conduction velocity spectrum of spontaneously active axons from saphenous nerves in diabetic BB/Wistar rats. With the exception of one or two fibers, all spontaneously active axons represented C-fibers (<3 m/s).

contained one or two spontaneously active fibers. Each filament was cut from the nerve after recordings were made from it.

Neural activity was amplified by a Grass P511 amplifier, (bandpass: 0.1 –10,000 Hz, Grass Instrument Co., Quincy, Massachusetts) and fed onto an audiomonitor and Tektronix 5113 and 565 oscilloscopes (Tektronik, Inc., Beaverton, Oregon). Data were recorded on an 8-channel FM (IRIG) tape recorder. Measurements of each fiber's spontaneous rate of discharge and conduction velocity were made. Stimuli were 0.1 or 1 ms monophasic pulses generated with a Grass S88 stimulator with a Grass PSIU6 constant-current stimulus isolation unit. The discharging axon was considered identified when an all-or-nothing action potential (AP) response identical in shape, amplitude, and duration to the spontaneous AP's was elicited at a fixed latency after stimulation of the proximal saphenous nerve. Conduction velocity was calculated by dividing the latency (minus a utilization time of 0.05 ms) by the length of the nerve between the stimulating and recording electrodes, as measured at the end of the experiment. A thorough survey of each saphenous nerve was made and each spontaneously discharging fiber was recorded. At the conclusion of recording, animals were killed by a lethal dosage of intravenous pentobarbital, and the saphenous nerves from both the recorded and unrecorded sides were removed for histologic analysis. For quantitative determination of the axonal population in diabetic nerves, planimetric measurements of electromicrographs of the fasicular area of the saphenous nerve contralateral to microfilament recordings were carried out in three animals. All myelinated fibers were counted in these nerves. Unmyelinated axons, 6–7 frames measuring 20 × 30 μm, were counted and a number was extrapolated for the entire nerve area.[21]

RESULTS

In the 10 normal Wistar rats, spontaneous activity could be recorded from the proximal stump of the saphenous nerve. This activity presumably originated in the region of the DRG, and has been described in normal Sabra rats by Wall and Devor[11] and in the Sprague-Dawley strain by Burchiel.[14] This activity involved axons with conduction velocities in the range of 0.3–5 m/s, and was seen in 2–33 axons (14.36 ± 8.86) when one entire saphenous nerve was systematically surveyed.

In contrast, recordings from the proximal stump of nerves in diabetic animals showed a much higher incidence of spontaneous activity, with 10–48 (28.45 ± 10.72) spontaneously discharging axons per saphenous nerve (Figure 1). More interestingly, the conduction velocities of spontaneously active fibers were almost universally of unmyelinated (C) fibers, with only an occasional small myelinated (A-delta) axon detected (Figure 2). There was a highly significant difference between the number of expected spontaneously active axons in normal rats and that in diabetic Wistar rats ($t = 3.2$, $df = 21$, $P < 0.001$). Furthermore, the pattern of spontaneous discharges from saphenous axons in diabetic animals was significantly more likely to involve "burst" activity (defined as sequential AP's with interspike intervals of <20 ms) than that of normals (2.73 ± 2.79 and 1.82 ± 2.59 axons/nerve, respectively; $t = 2.676$, $df = 21$, $P < 0.01$). Therefore, in the diabetic animals there was a markedly increased incidence of spontaneous discharges, particularly in burst firing patterns. Fiber counts from three diabetic nerves showed 842 ± 62 myelinated and 5013 ± 1192 unmyelinated axons/nerve. This would indicate that spontaneous activity occurred in approximately 0.6% of the unmyelinated fiber population in diabetic nerves.

Thus far, recordings from the distal saphenous nerve, either as an isolated segment or in continuity with receptors, have shown no qualitative differences in spontaneous activity when diabetic rats were compared with normal rats. However, differences in firing patterns and the modalities observed might be subtle, and therefore emerge only with further careful quantitative analysis.

DISCUSSION

Our preliminary results indicate that spontaneous discharges in unmyelinated afferents are a relatively common occurrence in saphenous nerves from diabetic rats. These discharges most probably originate in the region of the DRG and propagate both centrally and peripherally. If supported by more extensive studies, these results would have important implications for the understanding of diabetic neuropathic pain.

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