Hypersensitivity of Spinothalamic Tract Neurons Associated With Diabetic Neuropathic Pain in Rats

SHAO-RUI CHEN1 AND HUI-LIN PAN1,2
1Department of Anesthesiology and 2Department of Neuroscience and Anatomy, Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033-0850

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Chen, Shao-Rui, and Hui-Lin Pan. Hypersensitivity of spinothalamic tract neurons associated with diabetic neuropathic pain in rats. J Neurophysiol 87: 2726–2733, 2002; 10.1152/jn.00607.2001. Diabetic neuropathic pain is often considered to be caused by peripheral neuropathy. The involvement of the CNS in this pathological condition has not been well documented. Development of hypersensitivity of spinal dorsal horn neurons is involved in neuropathic pain induced by traumatic nerve injury. In the present study, we determined the functional changes of identified spinothalamic tract (STT) neurons and their correlation to diabetic neuropathic pain. Diabetes was induced in rats by intraperitoneal injection of streptozotocin. Hyperalgesia and allodynia were assessed by the withdrawal responses to pressure, radiant heat, and von Frey filaments applied to the hindpaw. Single-unit activity of STT neurons was recorded from the lumbar spinal cord in anesthetized rats. The responses of STT neurons to mechanical and thermal stimuli and the sensitivity to intravenous morphine were determined in diabetic and normal rats. In 12 diabetic rats, mechanical allodynia and hyperalgesia, but not thermal hyperalgesia, developed within 2 wk after streptozotocin injection and lasted for ≥7 wk. Compared to the 32 STT neurons recorded in normal animals, the 37 STT neurons in diabetic rats displayed a higher spontaneous discharge activity and enlarged receptive fields. Also, the STT neurons in diabetic rats exhibited lower thresholds and augmented responses to mechanical stimulation. Intravenous injection of 2.5 mg/kg of morphine suppressed significantly the responses of STT neurons to noxious stimuli in 12 nondiabetic rats. However, such an inhibitory effect of morphine on the evoked response of STT neurons was diminished in 14 diabetic animals. This electrophysiological study provides new information that development of hypersensitivity of spinal dorsal horn projection neurons may be closely related to neuropathic pain symptoms caused by diabetes. Furthermore, the attenuated inhibitory effects of morphine on evoked responses of STT neurons in diabetes likely accounts for its reduced analgesic efficacy in this clinical form of neuropathic pain.

INTRODUCTION

Diabetic neuropathy is one of the most common late complications of diabetes mellitus and is frequently painful, with the pain involving predominantly the distal extremities (Brown and Asbury 1984; Clark and Lee 1995). Neuropathic symptoms usually begin with tingling or burning sensations, particularly in the calves, ankles, and feet. Pain associated with diabetic neuropathy can occur either spontaneously or as a result of exposure to only mildly painful stimuli (hyperalgesia) or to stimuli not normally perceived as painful (allodynia) (Brown and Asbury 1984; Clark and Lee 1995). Pain caused by diabetic neuropathy is debilitating and often is resistant to opioid treatment (Clark and Lee 1995; Wright 1994). Although diabetic neuropathy is one of the most common etiologies of chronic pain in patients, the underlying mechanisms of persistent pain in diabetic patients remain poorly understood.

Diabetic neuropathic pain is widely regarded to be caused by peripheral neuropathy. It has been suggested that diabetic neuropathic pain results from hyperactivity of damaged small diameter C-fibers (Burchiel et al. 1985; Chen and Levine 2001). In this regard, electrophysiological studies have shown a significantly higher incidence of spontaneous discharges in comparison to normal nerves, and this spontaneous hyperactivity occurs almost exclusively in potentially nociceptive C-fibers (Burchiel et al. 1985; Chen and Levine 2001). Furthermore, C-fiber spontaneous discharges, mediated in part by protein kinase C activity, contribute to hyperalgesia caused by diabetic neuropathy (Ahlgren and Levine 1994). The mechanisms underlying diabetic neuropathic pain are complex, and both peripheral and central components of the sensory systems are likely involved. In addition to abnormalities of peripheral afferent nerves, altered sensory processing in the spinal cord may contribute to the development of diabetic neuropathic pain. However, most published studies on the functional changes of spinal dorsal horn neurons have focused on neuropathic pain caused by traumatic nerve injury (Chapman et al. 1998; Laird and Bennett 1993; Palecek et al. 1992; Takaishi et al. 1996).

The spinothalamic tract (STT) neurons represent a major ascending nociceptive pathway, and their contributions to neuropathic pain syndromes have been demonstrated in rats subjected to nerve ligation (Palecek et al. 1992). Because the etiology and mechanisms of diabetic neuropathic pain differ from those induced by nerve ligation, there is a strong need to investigate the possible plasticity of sensory neurons in the spinal cord and their role in the development of chronic pain in diabetic neuropathy. In the present study, we studied functional changes of spinal STT neurons and their possible correlations to hyperalgesia and allodynia in a rat model of diabetic neuropathic pain. Furthermore, although clinical and animal studies have shown a lack of analgesic effect of morphine on...
neuropathic pain (Arner and Meyerson 1988; Brown and Asbury 1984; Malcangio and Tomlinson 1998), altered responses of spinal STT neurons to opioids have not been studied in any neuropathic pain models. Thus we also tested a hypothesis that the inhibitory effect of morphine on the spinal STT neurons is reduced in diabetes.

METHODS

Induction of diabetes

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) initially weighing 225–250 g were used in this study. The surgical preparations and experimental protocols were approved by the Animal Care and Use Committee of the Penn State University College of Medicine. Diabetes was induced by a single intraperitoneal injection of 50 mg/kg of streptozotocin (STZ, Sigma Chemicals, St. Louis, MO) freshly dissolved in 0.9% sterile saline (Calcutt and Chaplan 1997; Courteix et al. 1993, 1994). Two weeks later, diabetes was confirmed in STZ-injected rats by measuring plasma glucose concentrations (>350 mg/dl) in blood samples obtained from the tail vein. The glucose level was assayed enzymatically using the Sigma diagnostic glucose reagents, and the colorimetric absorbance readings were performed at 450 nm using a micropate spectrophotometer (SPECTRaman Plus, Molecular Devices, Sunnyvale, CA). This experimental model of diabetic neuropathic pain has been described as a relevant model of chronic pain with alterations of pain sensitivity and poor responses to opioid treatment (Calcutt and Chaplan 1997; Courteix et al. 1993, 1994).

Behavioral testing

MECHANICAL HYPERALGESIA. Nociceptive mechanical thresholds, expressed in grams, were measured with a Ugo Basile Analgesimeter (Varese, Italy). The test was performed by applying a noxious pressure to the hindpaw. By pressing a pedal that activated a motor, the force increased (32 g/s) on a linear scale. When the animal displayed pain by withdrawal of the paw or vocalization, the pedal was immediately released and the nociceptive pain threshold read on a scale (the cutoff was 300 g to avoid tissue injury) (Courteix et al. 1994). Both hindpaws were used for assessment of mechanical hyperalgesia. At least two trials, separated by 10 min, were performed in each rat, and the mean value was used.

TACTILE ALLODYNIA. To quantify mechanical sensitivity of the hindpaw, rats were placed in individual plastic boxes on a mesh floor and allowed to acclimate for 30–45 min. A series of calibrated von Frey filaments (Stoeling, Wood Dale, IL) were applied perpendicularly to the plantar surface of the hindpaw with sufficient force to bend the filaments for 6 s. Brisk withdrawal or paw flinching were considered as positive responses. In the absence of a response, the filament of next greater force was applied. In the presence of a response, the filament of next lower force was applied. The tactile stimulus producing a 50% likelihood of withdrawal was determined using the “up-down” calculating method as described previously (Pan et al. 1999). Each trial was repeated two to three times at ~2-min intervals, and the mean value was used as the force to produce withdrawal responses.

THERMAL HYPERALGESIA. To quantitatively assess the thermal threshold of the hindpaw, rats were placed on the glass surface of a thermal testing apparatus (Model 336, IITC/Life Science Instruments, Woodland Hills, CA). The rats were allowed to acclimate for 30 min before testing. The temperature of the glass surface was maintained constant at 30°C. A mobile radiant heat source located under the glass was focused onto the hindpaw of each rat. The paw withdrawal latency was recorded by a digital timer. The withdrawal latency of both hindpaws from two to three consecutive trials was averaged, and the mean value was used as the thermal threshold. The cutoff of 30 s was used to prevent potential tissue damage (Chen and Pan 2001).

Electrophysiological recordings

Electrophysiological recordings were performed in diabetic rats 4–5 wk after STZ injection and in age-matched normal rats. Rats were anesthetized with intraperitoneal injection of pentobarbital sodium (50–60 mg/kg). The left carotid artery and jugular vein were cannulated for continuous blood pressure monitoring and for administration of additional doses of pentobarbital (10–20 mg/kg iv), respectively. Anesthesia was kept at a sufficient level as judged by the absence of corneal reflexes, withdrawal reflexes, and spontaneous blood pressure fluctuations. The trachea was cannulated, and the rat was ventilated mechanically. Arterial blood gases were analyzed with a blood gas analyzer and maintained within physiological limits. Throughout the experiment, body temperature was maintained in the range of 37–38°C with a circulating-water heating pad and heat lamps. Rats were placed in a stereotaxic frame (David-Kopf Instruments, Tujunga, CA), and the head and vertebral columns were rigidly fixed with proximal and distal vertebral clamps. A small hole was drilled on both sides of the skull to allow placement of a bipolar stimulating electrode (David-Kopf Instruments) in the ventral posterior lateral nucleus of the thalamus. The stimulating electrode was positioned according to the following stereotaxic coordinates using Bregma and brain surface as references: AP, 3.0–3.5 mm; ML, 3.0–3.2 mm; DV, 6–7 mm (Palecek et al. 1992). A limited laminectomy was performed to expose the spinal cord between L1 and L3 levels. The exposed brain and spinal cord tissues were covered with warm mineral oil. Extracellular recordings of STT dorsal horn neurons were obtained with glass pipettes filled with 5% KCl (impedance: 10–15 MΩ) that were descended into the spinal cord near the dorsal root entry zone up to a depth of 1,000 μm by a hydraulic microdrive (Stoeling) (Li and Pan 2000). Single-unit activity of STT neurons was recorded from the spinal dorsal horn neurons activated antidromically by electrical stimulation of the ventral posterior lateral nucleus of the thalamus. The action potential of the neuron was amplified, filtered with a band-pass filter (DAM 80, World Precision Instruments, Sarasota, FL), and processed through an audio amplifier and a storage oscilloscope (TDS 210, Tektronix, Wilsonville, OR). The neuronal impulse activity was recorded into a Pentium computer through an A/D interface board for subsequent off-line quantitative analysis. Discharge frequency was counted by using a data acquisition and analysis software (Experimental Workbench, DataWave Technology, Longmont, CO). When necessary, accurate counting of the single-unit discharge frequency was verified by comparing the constructed histogram with the hard copy recorded on a K2G thermal recorder (Astro-Med, W. Warwick, RI). At the end of the experiments, the rat was killed by intravenous injection of an overdose of pentobarbital (200 mg/kg).

Spinal STT neurons were searched by electrical stimulation (0.5 mA, 0.2 ms, 0.8–1.0 Hz; S48 Stimulator, Grass Instruments, Quincy, MA) through the stimulating electrode placed in the contralateral ventral posterior lateral nucleus of the thalamus (Hyliden and Wilcox 1986; Palecek et al. 1992). The spinal dorsal horn neurons were considered to be antidromically activated if the antidromically evoked spikes occurred at an invariant latency, the antidromically evoked spikes followed high-frequency stimulation (300–500 Hz), and the orthodromically elicited action potentials collided with antidromic spikes within the critical interval. Single-unit activity of STT neurons was isolated using a software window discriminator (DataWave Technology). The depth of the neuron from the surface of the dorsal horn of the spinal cord was recorded. After the receptive field was located and marked, the responses of STT neurons to the following mechanical stimuli were initially tested: brushing—the skin with a camel’s hair brush for 3–4 back-and-forth cycles, press—applying an intense pressure (~200 g/mm²) with the wooden tip of a cotton applicator perpendicularly to the skin for 5–6 s, which was perceived...
as mildly painful on the investigator’s skin, and pinch—a small forceps with a strong grip (~560 g/mm²) was applied to the marked receptive field (~560 g/mm²) for 5–6 s, which was perceived as distinctively painful in humans but not damaging to the skin. To calculate the frequency of response to each stimulus, the mean background activity occurring 10 s before the onset of the first stimulus was subtracted from the mean firing frequency that occurred during each stimulus. The STT neurons were divided into the following three categories according to their differential responses to mechanical stimulation: low-threshold (LT) neurons: cells responding maximally to brush (only showing rapid adapting responses to press and pinch at the beginning of the application); high-threshold (HT) neurons: neurons responding only to noxious pinch; and wide-dynamic-range (WDR) neurons: cells responding to brush but responding more intensely to noxious stimuli (pinch > press).

Furthermore, the response of STT neurons to mechanical stimulation of the receptive field with calibrated von Frey filaments of different bending forces (0.6, 1.0, 2.0, 4.0, 6.0, 8.0, 15, 26, and 60 g) was examined. The mechanical threshold was defined as the lowest force that caused either activation of the cell if no spontaneous activity was present or an increase in cell activity by ≥2 SD above the background activity. After determining the mechanical threshold of a STT cell with von Frey filaments, the filament of next greater force was used to map the size of the receptive field. The perimeters of skin areas responding to von Frey filament stimulation were outlined on a scale drawing of the hindpaw. The drawings were then scanned into the computer, and the areas of receptive fields were measured with an imaging analysis software (Analytical Imaging Station, Imaging Research, Canada). Additionally, the threshold of STT neurons in response to heat stimulation was measured using a feedback-controlled Peltier thermode with an active area of 36 mm² applied to the receptive field (Hylden and Wilcox 1986). The reference temperature was set at 38°C, and cycles of 5-s heat stimuli at 40, 42, 44, 46, 48, and 50°C were delivered in an ascending order to identify the thermal threshold for each STT cell. The probe temperature was allowed to return to baseline between successive stimuli. To determine the mean response frequency to thermal stimuli, the mean background activity occurring 10 s before each stimulus was subtracted from the mean frequency that occurred during the stimulus. The thermal threshold was defined as the lowest temperature that caused either activation of the STT neuron if no spontaneous activity was present, or an increase in cell activity by ≥2 SD above the background activity. Two to three STT neurons were typically studied in each rat.

Separate diabetic and normal rats were used to determine the inhibitory effect of morphine on identified STT neurons. Because morphine does not affect the response of LT spinal STT neurons even in normal rats (Hylden and Wilcox 1986), only WDR and HT STT neurons in diabetic and nondiabetic rats were recorded to test the effect of morphine. The responses of STT neurons to graded mechanical stimuli (brush, press, and pinch) and the thresholds of activation by von Frey filaments and the thermal stimulation were tested before and 15 min after intravenous injection of 2.5 mg/kg of morphine (Astra Pharmaceuticals, Westborough, MA). In some diabetic rats, 5 mg/kg of morphine was injected 30 min after examining the effect of 2.5 mg/kg of morphine on STT neurons. Only one STT neuron in each rat was studied in this protocol to examine the effect of morphine. The analgesic effect of this dose of morphine has been determined previously in normal conscious rats (Chen and Pan 2001).

At the end of the electrophysiological experiment, the location of the stimulating electrode was marked by passing DC current (50 μA for 30 s) at the site of the electrode tip. After the lesion was made, the rat was given a lethal injection of pentobarbital, and the brain was removed and fixed in 10% buffered formalin. Frozen sections were cut in 40-μm coronal sections on a freezing microtome (Leica), then mounted on slides and stained with cresyl violet (Sigma Chemical). The lesion sites of the thalamus were identified microscopically under brightfield illumination, reconstructed with the aid of a drawing tube, and plotted on standardized rat brain sections from the Paxinos’s atlas as we described previously (Li and Pan 2000).

Data analysis

Results are presented as means ± SEM. Significant changes in the withdrawal threshold in response to mechanical and thermal stimuli were determined using repeated-measures ANOVA followed by Dunnett’s post hoc test. The baseline discharge rate of STT neurons was averaged during control and the evoked responses were quantified as the mean discharge rate over the duration of the stimulus after subtracting the background activity of STT neurons. Comparisons between the spontaneous activity and evoked responses of STT neurons, the size of the receptive field, and the inhibitory effect of morphine on the evoked response of STT neurons in the control and diabetic groups were made by either Student’s paired t-test or repeated-measures ANOVA followed by Tukey’s post hoc test. Differences were considered to be statistically significant when \( P < 0.05 \).

RESULTS

Behavioral assessment

Rats developed hyperglycemia within 2 wk after STZ injection. The diabetic rats displayed polyuria, a reduced growth rate, and a marked increase in food and water intake. The paw withdrawal threshold measured with von Frey filaments before STZ treatment was 22.2 ± 2.6 g in 12 rats used for the behavioral study. The mechanical threshold in response to application of von Frey filaments decreased significantly (6.4 ± 1.5 g, \( P < 0.05 \)) 2 wk after STZ injection and lasted for ≥7 wk (Fig. 1A). The withdrawal threshold in response to the pressure applied to the hindpaw was also decreased significantly during this time course in 12 diabetic rats (Fig. 1B). However, the withdrawal latency in response to the noxious heat stimulus was not changed significantly in these diabetic rats ≤7 wk after STZ injection (Fig. 1C).

Electrophysiological recordings

A total of 18 diabetic (\( n = 37 \) cells) and 16 normal (\( n = 32 \) cells) rats was used for the electrophysiological recordings of STT neurons. Figure 2 shows the locations of the tip of the stimulating electrode in the ventral posterior lateral nucleus of thalamus in all normal and diabetic rats used for electrophysiological experiments. A representative histology section showing the stimulation and lesion site in the ventral posterior lateral nucleus of thalamus in one diabetic rat is presented in Fig. 3. Data from three normal and five diabetic rats were excluded from analysis because the lesion site was not located within the ventral posterior lateral nucleus of thalamus. The STT neurons recorded had a mean depth of 574 ± 26 and 612 ± 34 μm (\( P > 0.05 \)) in diabetic and normal rats, respectively. The antidromic latency (5.2 ± 1.1 vs. 4.6 ± 0.8 ms) of stimulation of STT neurons was similar in diabetic and normal rats. Figure 4 are original tracings showing an antidromically activated STT neuron using the criteria described in the Methods. In 34 STT cells (23 in diabetic and 11 in normal group) examined for the antidromic activation threshold, the lowest current required for antidromic activation ranged from 6 to 23 μA (18.1 ± 1.4 μA).

SPONTANEOUS DISCHARGERS AND RECEPTIVE FIELDS OF STT NEURONS. The baseline spontaneous activity of STT neurons in diabetic rats was significantly higher than that in normal rats.
While only 6% of STT neurons in normal rats had a background activity (>5 imp/s, many (32%) STT neurons in the diabetic group displayed a high spontaneous baseline activity (>5 imp/s, Fig. 5A). A majority of the STT neurons in both groups had a receptive field on the glabrous skin of the hindpaw. The size of the receptive field of 37 STT cells in diabetic rats was 123.8 ± 22.7 mm², which was significantly larger than that of 32 STT neurons recorded in normal animals (48.6 ± 13.4 mm²).

RESPONSES OF STT NEURONS TO MECHANICAL AND THERMAL STIMULI. The distribution of STT cells in normal and diabetic rats is summarized in Fig. 6. In normal rats, most STT (87.5%) neurons were classified as HT neurons; only 3 of 32 (9.3%) STT neurons were WDR neurons. However, in diabetic rats, 18 of 37 (48.6%) STT neurons were WDR neurons, while 13 of 37 (35.1%) STT neurons were categorized as HT neurons. Also, 6 of 37 (16.2%) STT neurons were LT neurons in the diabetic group. Compared to those recorded in normal rats, the STT neurons in diabetic rats showed an increased response to graded mechanical stimulation (brush, press, and pinch; Figs. 7 and 8A). The STT neurons in the diabetic group had a significantly lower threshold (Fig. 9A) and augmented responses to application of von Frey filaments (Fig. 8B). After correcting for spontaneous baseline activity, the mechanical responses of STT neurons in diabetic rats were still significantly greater than those in normal rats. However, the thermal threshold of STT neurons was similar in diabetic and normal animals (Fig. 9B).

EFFECT OF MORPHINE ON EVOKED RESPONSES OF STT NEURONS. The effect of intravenous injection of 2.5 mg/kg of morphine on STT neurons was studied in 14 diabetic and 12 normal rats. There were eight WDR and six HT neurons in the diabetic group. Four WDR and eight HT neurons were studied in the normal group. In normal rats, the responses of STT neurons to press and pinch were suppressed significantly by intravenous injection of morphine (Fig. 10A). Such inhibitory effect lasted for 40–45 min after morphine injection, which matched the analgesic effect of morphine in conscious rats (Chen and Pan 2001). However, the inhibitory effect of 2.5 mg/kg morphine
on the evoked responses of STT neurons to mechanical stimuli in diabetic rats was diminished (Fig. 10B). In 8 of 14 STT neurons in diabetic rats, further injection of 5 mg/kg morphine only partially suppressed the response of STT neurons to mechanical stimulation diminished (Fig. 10B). We noted that the evoked responses of neither WDR nor HT neurons were significantly suppressed by morphine in the diabetic group, and thus the data from these two types of STT neurons were pooled. Intravenous morphine also increased significantly the activation threshold of STT neurons in response to thermal stimulation (from 46.8 ± 1.2 to 50.3 ± 1.6°C, $P < 0.05$) and von Frey filaments (from 22.44 ± 0.77 to 58.37 ± 0.84 g, $P < 0.05$) in normal rats. In contrast, intravenous morphine only slightly increased the threshold of STT neurons in response to thermal stimulation (from 46.2 ± 1.5 to 48.8 ± 1.8°C, $P > 0.05$) and von Frey filaments (from 8.14 ± 0.67 to 9.62 ± 0.87 g, $P > 0.05$) in diabetic rats.

**DISCUSSION**

This is the first study demonstrating the presence of hyper-sensitivity of spinal ascending projection neurons and their correlation to the symptoms of diabetic neuropathic pain. Because the STT neurons are important ascending nociceptive pathways, we determined the potential functional changes of these neurons and their relevance to the neuropathic pain state in diabetes in the present study. There are two important findings in the current study. First, we found that spinal STT
neurons in diabetic rats exhibited an increased background activity, enlarged receptive field, and augmented responses to mechanical stimuli, which match the behavioral data in this model. Furthermore, we found that the inhibitory effect of morphine on spinal STT neurons in normal animals was reduced in diabetic rats, which is in agreement with the experimental and clinical data showing reduced opioid analgesic effect on neuropathic pain in diabetes. Thus this electrophysiological study provides new information about the altered sensitivity of spinal STT neurons and their potential role in the development of chronic pain caused by diabetic neuropathy.

Diabetic neuropathic pain is often chronic and difficult to manage because it is resistant to classical analgesics. The underlying mechanisms for persistent pain and resistance to opioid treatment in diabetic patients are not fully known. In this study, we first systematically examined the time course of development of symptoms related to neuropathic pain following STZ injection in rats. Consistent with previous studies (Courteix et al. 1993; Malcangio and Tomlinson 1998), the prominent feature of this model of diabetic neuropathic pain is the presence of significant mechanical hypersensitivity. Mechanical allodynia and hyperalgesia, assessed with application of von Frey filaments and noxious pressure to the hindpaw, progressively developed within 2 wk after STZ injection. We found that these conditions were maintained for ≥7 wk in diabetic rats. However, we did not observe significant changes in the withdrawal latency to noxious heat stimulation ≤7 wk in diabetic rats. Reports of changes in thermal nociceptive thresholds in this model have been highly variable, with thermal

FIG. 8. Differential responses of 32 normal and 37 diabetic STT neurons to stimulation of the receptive field with graded mechanical stimuli (A) and von Frey filaments (B). *P < 0.05 compared with the respective controls.

FIG. 9. Changes in the stimulation threshold of 32 normal and 37 diabetic STT neurons determined by von Frey filaments (A) and the radiant heat stimulation (B). *P < 0.05, compared with the values in the normal group.

FIG. 10. Comparison of the inhibitory effect of intravenous injection of morphine on the responses to the graded mechanical stimulation of 12 normal (A) and 14 diabetic (B) STT neurons. Note that 8 of 14 STT neurons in diabetic rats were tested for the effect of 5 mg/kg of morphine. *P < 0.05, compared with the same intensity of stimulation before morphine treatment.
hyperalgesia observed in some studies (Courteix et al. 1993) and others observing no changes (Raz et al. 1988) or decreased thermal sensitivity (Malcangio and Tomlinson 1998; Pertovaara et al. 2001). Because thermal nociception is mainly transmitted through unmyelinated C-fiber afferents (Ossipov et al. 1999), it seems possible that the C-fiber afferents mediating thermal nociception are still largely preserved, at least during this period, in this rat model of diabetic neuropathic pain. Some limitations for the use of radiant heat stimuli to detect thermal hyperalgesia must be recognized because it only measures the noxious thermal detection threshold. It remains to be determined if the thermal sensitivity to suprathreshold stimuli or duration responses to noxious heat stimulation are altered in this rat model of diabetic neuropathic pain.

Development of mechanical hypersensitivity (allodynia and hyperalgesia) in diabetic rats could be due to hyperexcitability of spinal dorsal horn neurons in response to mechanical stimuli. Although diabetic neuropathy is a major etiology of chronic neuropathic pain in patients, the contribution of spinal dorsal horn neurons to neuropathic pain in diabetes has not been well recognized. The potential role of spinal dorsal horn neurons in diabetic neuropathic pain has been implicated in a recent report, although it cannot be determined whether the dorsal horn neurons recorded are interneurons or projecting neurons (Pertovaara et al. 2001). The main finding by Pertovaara et al. is that spinal dorsal horn neurons have a high spontaneous activity in diabetic rats (Pertovaara et al. 2001). To further explore the contribution of spinal dorsal horn neurons to diabetic neuropathic pain, we focused our study specifically on functional alterations of spinal STT neurons in diabetic rats. We chose to examine the functional changes of STT neurons 4–5 wk after STZ injection because stable allodynia and hyperalgesia were developed in this period. In the present study, we found that STT neurons in diabetic rats manifested hyperexcitability similar to those reported in nerve ligation models (Chapman et al. 1998; Laird and Bennett 1993; Palecek et al. 1992; Takaishi et al. 1996). The most striking features of functional changes of STT neurons are the marked increase in the spontaneous discharge activity and the augmented responses of STT neurons to mechanical stimuli. Also, a higher proportion of STT neurons exhibited abnormal levels of elevated spontaneous activity in diabetic animals. The decreased threshold and augmented response of STT neurons to mechanical stimulation are consistent with the presence of mechanical hypersensitivity observed in these diabetic animals. Interestingly, the size of the receptive field of STT neurons in diabetic rats was significantly larger than that of nondiabetic rats; this has been reported in dorsal horn neurons of rats subjected to sciatic nerve ligation (Takaishi et al. 1996). Compared to that of normal rats, the threshold of STT neurons in response to the thermal stimulation was not altered significantly in diabetic rats. Taken together, the abnormal hypersensitivity of dorsal horn STT neurons could constitute a functional basis for the altered mechanical sensitivity exhibited in diabetic rats and may play an important role in the maintenance of persistent pain in diabetes.

Although the mechanisms of increased excitability of spinal dorsal horn neurons in diabetic rats are not fully known, the development of the hypersensitivity of STT neurons may be due to the presence of ectopic discharges from primary afferent nerves reported in diabetic animals (Burchiel et al. 1985; Chen and Levine 2001). For example, the origin of high-frequency discharge activity of spinal dorsal horn neurons induced by nerve injury has been considered to originate from the ectopic discharges at the injury site, and the increased level of the spinal neurons’ excitability is secondary to the generation of afferent ectopic discharges at the nerve injury site (Gracely et al. 1992; Laird and Bennett 1993). We found that STT neurons in diabetic rats displayed hypersensitivity to mechanical stimuli, suggesting that the increased responsiveness of the STT neurons is likely due to increased responsiveness of the primary afferents. It is likely that aberrant activity generated in the afferent nerves contributes to the status of hypersensitivity of spinal dorsal horn neurons developed in diabetes. Rewiring of dorsal horn neurons may also contribute to the altered sensory symptoms in diabetic rats. In this regard, it has been demonstrated that peripheral nerve injury can induce structural reorganization leading to sprouting of myelinated afferents into spinal lamina II (Woolf et al. 1992). Other factors, such as upregulation of spinal excitatory glutamate receptors and increased release of glutamate and substance P, may be important for the development of spinal hypersensitivity in diabetes (Dougherty and Willis 1991; Dougherty et al. 1992; Harris et al. 1996). In this regard, peripheral nerve injuries increase the expression of N-methyl-D-aspartate (NMDA) and non-NMDA receptors in the dorsal spinal cord (Harris et al. 1996). Microiontophoresis of glutamate receptor agonists to the spinal cord causes the hypersensitivity of dorsal horn neurons, including increased background discharge frequency and augmented responses to mechanical stimulation of skin (Dougherty and Willis 1991; Dougherty et al. 1992), which likely is the functional basis of allodynia and hyperalgesia in many animal models of chronic pain. Therefore both the peripheral and spinal plasticity of the somatosensory system may be important for the development of neuropathic pain in diabetes.

Another salient finding of the present study is that the inhibitory effect of systemic morphine on the responses of STT neurons to noxious stimulation is reduced in diabetic animals. One of the important sites for the analgesic action of morphine is achieved by its inhibitory effect on spinal dorsal horn neurons (Hylden and Wilcox 1986). The μ opioid receptors are located in the dorsal horn of the spinal cord (Schulz et al. 1998). It has been demonstrated that opioid-containing cells synapse on STT neurons (Ruda 1982) and administration of morphine inhibits spinal nociceptive STT neurons (Hylden and Wilcox 1986). We observed that intravenous morphine produced greater inhibitory effects on responses of STT neurons to noxious stimuli as compared with innocuous stimulation-evoked responses in normal rats. Clinical and experimental studies have consistently shown a reduced or lack of analgesic effect of morphine on diabetic neuropathic pain (Calcutt and Chaplan 1997; Courteix et al. 1994; Malcangio and Tomlinson 1998; Wright 1994). Consistent with these reports, we found that the inhibitory effect of intravenous morphine on STT neurons in response to mechanical and thermal stimuli was significantly attenuated in diabetic rats. The reason for the reduced inhibitory effect of morphine on spinal STT neurons in diabetic rats is not clear. It may be due to loss or downregulation of μ opioid receptors in the spinal cord. It is also possible that the descending inhibitory control or the signal transduction systems activated by morphine may be impaired in diabetic neuropathic pain. However, there is no substantial
evidence to support this hypothesis at this time. The results of the current investigation provide an important basis to further explore the neurophysiological mechanisms of diabetic neuropathic pain.

In summary, our electrophysiological study provides substantial evidence that there are functional changes of spinal STT neurons following induction of diabetes in rats. The present study provides important information that, in addition to the component of peripheral afferent nerves, the dorsal horn STT neurons also likely play an important role in chronic neuropathic pain in diabetes. The presence of hypersensitivity of spinal STT neurons may be causally related to the neuropathic pain state in diabetes. The finding that the inhibitory effect of morphine on the spinal projecting neurons is attenuated in diabetic rats provides an important insight for the reduced analgesic action of opioids on diabetic neuropathic pain.

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REFERENCES


Dougherty PM and Willis WD. Enhancement of spinothalamic neuron responses to chemical and mechanical stimuli following combined microiontophoretic application of N-methyl-D-aspartic acid and substance P. Pain 47: 85–93, 1991.


