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CHARACTERIZATION OF UPPER THORACIC SPINAL NEURONS RESPONDING TO ESOPHAGEAL DISTENSION IN DIABETIC RATS

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Abstract

The aim of this study was to examine spinal neuronal processing of innocuous and noxious mechanical inputs from the esophagus in diabetic rats. Streptozotocin (50 mg/kg, ip) was used to induce diabetes in 15 male Sprague-Dawley rats, and vehicle (10 mM citrate buffer) was injected into 15 rats as control. Four to eleven weeks after injections, extracellular potentials of single thoracic (T3) spinal neurons were recorded in pentobarbital anesthetized, paralyzed, and ventilated rats. Esophageal distensions (ED, 0.2, 0.4 ml, 20s) were produced by water inflation of a latex balloon in the thoracic esophagus. Noxious ED (0.4 ml, 20 s) altered activity of 44% (55/126) and 38% (50/132) of spinal neurons in diabetic and control rats, respectively. The short-lasting excitatory responses to ED were encountered more frequently in diabetic rats (27/42 vs 15/41, $P < 0.05$). Spinal neurons with low threshold for excitatory responses to ED were more frequently encountered in diabetic rats (33/42 vs 23/41, $P < 0.05$). However, mean excitatory responses and duration of responses to noxious ED were significantly reduced for high-threshold neurons in diabetic rats (7.4 ± 1.1 vs 13.9 ± 3.3 imp/s; 19.0 ± 2.3 vs 31.2 ± 5.5 s; $P < 0.05$). In addition, more large size somatic receptive fields were found for spinal neurons with esophageal input in diabetic rats than in control rats (28/42 vs 19/45, $P < 0.05$). These results suggested that diabetes influenced response characteristics of thoracic spinal neurons receiving mechanical esophageal input, which might indicate an altered spinal viscerosensitive processing underlying diabetic esophageal neuropathy.

Keywords

Visceral pain; diabetes; autonomic neuropathy; spinal dorsal horn; extracellular recording

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INTRODUCTION

Esophageal motor and sensory dysfunctions occur frequently in patients with diabetic autonomic neuropathy. Disturbances of esophageal motor function include reduction in the amplitude of esophageal contraction, less numerous peristaltic waves, increased number of spontaneous and spastic contractions, decreased lower esophageal sphincter pressure, impaired esophageal transit, and gastroesophageal reflux (Ahmed et al. 2006; Hollis et al. 1977; Holloway et al. 1999; Kinekawa et al. 2001). These changes in esophageal motility also are associated with abnormal activity in the esophageal sensory afferent system. For example, diabetic patients show elevation of the perception threshold to esophageal electrical and mechanical stimulation (Rathmann et al. 1991; Kamath et al. 2000), decreased sensitivity to differentiated and standardized stimulation of the esophagus that is accompanied by an increase in the somatic referred pain (Frokjaer et al. 2007), and reduced amplitude, prolonged latency and an erratic non-reproducible response of evoked brain potentials to mechanical and electrical stimulation of the esophagus (Tougas et al. 1992; Kamath et al. 1998). A higher prevalence (28%) of abnormal gastroesophageal reflux also appears among asymptomatic diabetic patients than among the general population (Lluch et al. 1999). In addition, histomorphometric and biomechanical remodeling in the muscle and mucosa-submucosa layer of the diabetic esophagus are characterized by increased circumferential length, luminal area and circumferential stiffness (Yang et al. 2006; Zhao et al. 2007). These findings may represent an impaired esophageal wall resulting from changes in peripheral afferent and efferent pathways and/or the central integrative system of the spinal cord and brain.

Sensory information from the esophagus reaches the central nervous system via vagal and spinal visceral afferent fibers. In rats, the cervical and upper thoracic portions of the esophagus are innervated predominantly by afferent fibers in the spinal visceral afferent and recurrent laryngeal nerves, whereas vagal afferent fibers originating in the myenteric plexus innervate the lower thoracic or abdominal esophagus (Neuhuber and Clerc 1990; Dütsch et al. 1998). It is believed that vagus nerve impairment may be responsible for abdominal esophageal function in diabetes mellitus (Kamath et al. 1998; Smith 1974; Tougas et al. 1992). However, spinal transmission and integration of sensory inputs in diabetes have been studied primarily for somatic inputs, and little information is available for diabetic effects on spinal visceral inputs. Spinal afferent innervation of the esophagus extends from C1-L3 dorsal root ganglia, with different peak distributions in cervical and thoracic spinal cord in dogs, cats and rats (Hudson and Cummings 1985; Neuhuber and Clerc 1990; Dütsch et al. 1998). A few studies have suggested that the spinal dorsal horn, the first relay station of sensory afferent pathways, is affected by diabetes. For example, streptozotocin (STZ)-induced diabetic rats associated with somatic mechanical and thermal hyperalgesia, c-fos expression in dorsal horn spinal neurons of superficial and deeper laminae is significantly increased (Morgado and Tavares 2007), whereas STZ-induced chemogenic hypoalgesia in C57Bl/6 mice is associated with a reduced fos expression in the spinal cord (Johnson et al. 2007). Electrophysiological studies have shown higher spontaneous activity and greater responses of spinal dorsal horn neurons or spinothalamic tract neurons to innocuous or noxious somatic stimulation, and enlarged receptive fields also are observed in STZ-induced diabetic rats (Pertovaara et al. 2001; Chen and Pan et al. 2002). Enhanced spinal neuronal excitability and segmental motor output are partly because of decreased activity in spinal intrinsic inhibitory circuits (Tanabe et al. 2005) as well as impaired balance between facilitatory and inhibitory descending pathways (Kimura et al. 2005; Pertovaara et al. 2001). However, the above data are based on observations of intraspinal mechanisms underlying peripheral somatic stimuli in diabetic animals. Effects of esophageal stimulation on spinal neuronal processing have not been examined in diabetic animals.

We have shown that spinal dorsal horn neurons in the upper thoracic and cervical spinal cord respond to stimulation of thoracic mechanoreceptive afferents originating from esophagus in cats and rats (Garrison et al. 1992; Qin et al. 2003; 2004). Esophageal distension increases background activity in the majority of spinal neurons, and the remainder show inhibitory or biphasic response patterns. These esophageal receptive neurons are modulated by vagal afferents, esophageal inflammation, inputs from distant spinal segments, central respiratory drive, and also receive convergent inputs from somatic structures (Euchner-Wamser et al. 1993; Garrison et al. 1992; Qin et al. 2002; 2003; 2004). Furthermore, it also is reported that intraesophageal chemicals (e.g. HCl, bradykinin and capsaicin) enhance responsiveness of upper thoracic spinal neurons to mechanical stimulation of esophagus in rats (Qin et al. 2008). Therefore, these spinal neurons with esophageal inputs might modulate intraspinal esophagoreceptive processing as well as noxious visceromotor reflexes, and play an important role in esophageal pain and/or hypersensitivity (Jou et al. 2002; Lynn et al. 1992; Sengupta 2006). The purpose of this study was to quantitatively characterize responses of upper thoracic spinal neurons (T3) to esophageal distension and somatic stimuli in rats with STZ-induced diabetes. A preliminary report of this work has been presented in abstract form (Qin et al. 2007).

METHODS

Experiments were performed in 30 male Sprague-Dawley rats (Harlan Inc., USA) weighing between 280–400 g. Diabetes was induced by intraperitoneal administration of streptozotocin (50 mg/kg in 10 mM citrate buffer, pH 4.5, n=15). In another group of rats (n=15), citrate buffer was injected as control. Blood glucose and body weight were measured immediately before injection of streptozotocin or vehicle. Weekly measurements of blood glucose and body weight were performed in both of groups of animals after the injections. The protocol for this study was approved by the Institutional Animal Care and Use Committee at the University of Oklahoma Health Science Center.

The electrophysiological study was conducted 4–11 weeks after streptozotocin or vehicle injections. Initial anesthesia was induced with sodium pentobarbital (60 mg/kg, i.p.). During the experiment, a continuous intravenous infusion of pentobarbital (15–25 mg/kg/hr) was used to maintain the level of anesthesia. Catheters were inserted into the right carotid artery to monitor blood pressure and into the left jugular vein to inject fluids and drugs. A tracheotomy was performed for artificial ventilation using a constant-volume pump (55–60 strokes/min, 3.0–5.0 ml stroke volume). Animals were paralyzed initially with pancuronium bromide (0.4 mg/kg, i.p.) and supplemental doses (0.2 mg/kg/hr, i.p.) were administered to maintain muscle relaxation throughout the experiment. A thermostatically controlled heating pad and overhead infrared lamps were used to maintain rectal temperature between 36.7° and 37.3°C.

Laminectomies were performed to expose spinal segment T3 for recording activity of spinal neurons. Rats were mounted in a stereotaxic headholder and clamped at T2 and T6–T8 vertebrae. Dura mater was carefully removed and the spinal cord was covered with warm agar (3–4% in saline) to improve stability. Carbon-filament glass microelectrodes were used to record extracellular action potentials of single spinal neurons in the T3 segment. All recordings were made 0–2 mm lateral from midline and at a depth of 0–1.2 mm from dorsal surface of the spinal cord. Extracellular potentials were fed into a window discriminator, displayed on an oscilloscope, and stored in a computer with Spike 3 data acquisition software (CED, Cambridge) for off-line analyses. Neuronal activity was measured using rate histograms (1 s/bin). Spontaneous activity of neurons was determined by counting activity for 10 s before a stimulus to obtain impulses per second (imp/s). Neuronal responses (imp/s) during stimuli were defined as increases or decreases $\geq 20\%$ in maximal activity compared to spontaneous activity. For neurons with a firing rate < 5 Hz, a response was considered valid only if activity increased

or decreased by ≥ 1 Hz. Statistical comparisons were made using Student's paired or unpaired *t*-test and Chi-square analysis. Data are presented as means $\pm$ S.E and $P < 0.05$ was considered statistically significant.

Esophageal distension (ED) was produced by infusing warm water to inflate a small latex balloon (1.0 cm in length) at the end of PE-240 tubing. Balloons were inserted perorally 7–10 cm from the front incisors for thoracic ED (Qin et al. 2003). The tubing that extended from the mouth was attached to the stereotaxic frame with adhesive tape to avoid movement of the balloon during repeated distensions. ED was produced by injection of warm water (0.2, 0.4 ml at a rate of 0.05–0.1 ml/s and held for 20s) in an esophageal balloon. These volumes have been used as natural stimuli in studies of behavioral mechanisms and central neural processing of esophageal reflexes in rats (Dong et al. 2001; Qin et al. 2003; 2004; Wei et al. 1997). It should be noted that ED was used as the research stimulus to identify spinal neurons responding to ED. The low volume ED (≤ 0.2 ml) is considered to be an innocuous distension, whereas ED ≥ 0.4 ml is likely to be noxious or painful in rats (Dong et al. 2001; Euchner-Wamser et al. 1993; Qin et al. 2003; 2004).

Cutaneous receptive fields of spinal neurons were tested for responses to innocuous brush with a camel-hair brush or noxious pinch of skin and muscles with a blunt forceps. Three types of neurons were categorized as follows: low-threshold (LT) neurons only responded to brushing hair; high-threshold (HT) neurons only responded to noxious pinching of the somatic field; wide dynamic range (WDR) neurons responded to brushing the hair and also had a greater response to noxious pinching of the somatic field. Areas of the somatic fields were measured and assigned to the following categories: small size (long axis ≤ 3 cm) and large size (> 3 cm). Outlines and descriptions of receptive fields were made for all neurons examined.

To mark spinal recording sites, an electrolytic lesion (50 μ A DC anodal for 20s, cathodal for 20s) was made at recording sites after study of a neuron that responded to ED. At the end of the experiment, animals were euthanized with an intravenous overdose of pentobarbital (200 mg/kg). The thoracic spinal cord was removed and placed in 10% buffered formalin solution. After ≥ 3 days, frozen sections (55–60 μ m) of the thoracic spinal cord were made and lesion sites in the dorsal gray matter were viewed and documented. Laminae were identified using the cytoarchitectonic scheme of Molander et al. (1989).

RESULTS

The animals with streptozotocin injection showed an increase of blood glucose within 2–3 days and maintained a high values compared to those in control animals with vehicle injection. On the day of the experiment, the average blood glucose value was 469.6 ± 24.9 mg/dl in the diabetic animals ($n = 15$), which is significantly higher than 104.7 ± 4.7 mg/dl in control animals ($n = 15$, $P < 0.01$). The body weight of animals was significantly lower in diabetic animals than control animals (262.1 ± 14.0 g vs 426.9 ± 9.0 g, $P < 0.01$) on the day of experiment. In this study, a total of 126 and 132 spinal neurons were characterized in diabetic and control groups of animals, respectively.

Response patterns and spontaneous activity

Noxious ED (0.4 ml, 20s) changed the activity in 55/126 (44%) spinal neurons recorded in diabetic rats and in 50/132 (38%) neurons recorded in control animals. Three patterns of neuronal responses to ED were classified: excitation, inhibition, and excitation-inhibition. Examples of these responses are shown in Fig. 1A–D. No significant differences were found between the proportions of ED-response patterns in diabetic and control animals (Fig. 1E). Spinal neurons excited by ED were divided into two groups based on their background activity: silent neurons with low spontaneous activity (≤ 0.5 imp/s) and active neurons with high

spontaneous activity (>0.5 imp/s). The ratio of silent and active neurons in diabetic rats was similar to that in control animals (5/37 vs 7/34). A comparison of the characteristics of spontaneous activity and ED-responses of spinal neurons from diabetic and control animals are presented in Table 1. The mean duration of excitatory responses to noxious ED was significantly shorter in spinal neurons recorded from diabetic rats than those recorded from control rats (25.9 ± 2.7 s vs 31.3 ± 3.1 s, $P < 0.05$).

Short- and long-lasting responses

Based on the recovery time of neuronal activity to the control level after termination of noxious ED (0.4 ml, 20s), neurons excited or inhibited by ED were further subdivided into two groups (Qin et al. 2003): neurons with recovery time ≤ 5 s were classified as short-lasting excitatory (SL-E, Fig. 1A), and neurons with recovery time > 5 s were classified as long-lasting excitatory (LL-E, Fig. 1B). The SL-E neurons were encountered more frequently in diabetic rats compared to control animals (27/42 vs 15/41, $P < 0.05$, Fig. 1F). Quantitative analyses of spontaneous activity, excitatory responses to ED, duration, and latency of responses in SL-E and LL-E neurons after noxious ED are shown in Table 2.

Low- and high-threshold responses

Based on the intraesophageal distending volume that produced a response, neurons excited by ED were divided into two subgroups: low-threshold (LT) neurons responded to ≤ 0.2 ml ED (20s); high-threshold (HT) neurons responded to ≥ 0.4 ml of ED (Qin et al. 2003). Examples of these neurons are shown in Fig. 2A, B. Spinal neurons in diabetic rats were more likely to have LT responses to ED than control rats (33/42 vs 23/41, $P < 0.05$, Fig. 2C). Comparisons of spontaneous activity and excitatory responses of low- and high-threshold spinal neurons to ED are shown in Table 3. Mean excitatory responses and duration of responses of HT neurons to noxious ED were significantly reduced in diabetic rats compared to control rats (7.4 ± 1.1 imp/s vs 13.9 ± 3.3 imp/s; 19.0 ± 2.3 s vs 31.2 ± 5.5 s; $P < 0.05$), respectively. Mean latency of excitatory responses of HT neurons was significantly longer in diabetic than in control rats (3.7 ± 1.0 s vs 2.9 ± 0.7 s, $P < 0.05$).

Location of ED-responsive neurons

Lesions made at the recording sites were identified histologically for 24 ED-responsive neurons in diabetic rats and for 27 neurons in control rats. The majority of neurons with esophageal inputs were located in laminae V, VI, VII and X, and no significant difference was found in the distribution of neurons responding to ED between diabetic and control groups (Fig 3A–C). Spinal neurons recorded at <0.3 mm in depth from the dorsal surface of spinal cord were classified as superficial neurons and at 0.3–1.2 mm were classified as deeper neurons (Qin et al. 2003;2004). Regional distribution of the recording sites of spinal neurons with different response patterns in superficial and deeper laminae of spinal cord in diabetic animals was not different from the control group (Fig.3D, E). A comparison of the characteristics of spontaneous activity and responses of superficial and deeper spinal neurons to ED is shown in Table 4.

Responses to somatic inputs

Examination of responses to somatic inputs showed that 45/55 (80%) neurons in diabetic rats and 47/50 (94%) neurons in the control group received convergent inputs from cutaneous receptive fields. Cutaneous receptive fields were generally on the chest, axilla, shoulder, and upper back areas. Fig. 4A–C shows three examples of neurons that received inputs from somatic fields (LT, WDR, and HT). No statistically significant differences in the proportions of somatic field properties of ED-responsive neurons between diabetic and control groups were observed (Fig. 4D). However, spinal neurons with large somatic receptive fields were

encountered more frequently in diabetic rats than in the control group (17/46 vs 28/47, $P < 0.05$, Fig. 4E).

DISCUSSION

To the best of our best knowledge, this is the first study to characterize activity of upper thoracic neurons with mechanical esophageal input in STZ-induced diabetic rats. Although the percentage (44%) of spinal neurons responding to noxious ED in diabetic rats was not significantly different from the percentage (38%) of neurons in control group, differences in response characteristics were observed in subgroups of spinal neurons. 1) SL-E neurons were encountered more frequently in diabetic rats compared to control animals. Thus, the mean duration of excitatory responses to noxious ED was significantly shorter in spinal neurons recorded from diabetic rats than in those recorded from the control group. 2) Spinal neurons in diabetic rats were more likely to have LT-E responses to ED than neurons in control rats. 3) For HT-E neurons, mean responses and duration to noxious ED were significantly reduced, while mean latency for responses was significantly longer in diabetic rats than in control rats. 4) Spinal viscerosomatic convergent neurons with large somatic receptive fields were encountered more frequently in diabetic rats than in the control group. These data suggested that altered discharge characteristics of upper thoracic spinal neurons in diabetic rats might contribute to diabetic autonomic neuropathy.

Spontaneous activity

In the present study, no significant differences in spontaneous activity were observed between diabetic and control rats in any subgroups of spinal neurons excited or inhibited by noxious ED. The ratio of silent and active neurons in diabetic rats also was similar to that in control animals. These findings are different from the reports in some previous studies. For example, in rats with STZ-induced diabetes, wide-dynamic range spinal neurons recorded in T12-L2 segments and spinothalamic tract (STT) neurons in L4–L5 segments had higher spontaneous activity than the normal groups (Pertovaara et al. 2001; Chen and Pan 2002). A higher number of Fos-immunoreactive neurons in T13-L3 spinal segments is reported in both superficial and deeper laminae of spinal dorsal horn in rats with STZ-induced diabetes than in control animals (Morgado and Tavares 2007). However, the location of the deeper neurons did not appear to be as deep as those recorded in this study. In general, viscerosomatic convergent neurons are located in laminae V–IX, whereas neurons with only somatic input are located primarily in laminae II–IV (Cervero 1994). It is also noted that the increased spontaneous activity in neurons of diabetic animals are associated with mechanical and/or thermal hypersensitivity of somatic receptive fields (Morgado and Tavares 2007). In contrast, the present study focused on characteristics of spinal neurons with visceral and/or viscerosomatic spinal inputs from esophagus rather than somatic input. Our search stimulus for spinal neurons responses was esophageal distension rather than somatic stimuli. Thus, neurons with visceral or viscerosomatic input might respond with different characteristics compared to neurons that responded to somatic input only.

SL-E and LL-E responses

The SL-E and LL-E spinal neuronal responses might correlate with the types of afferent fibers or receptors in the esophagus. For example, SL-E responses might result from activation of rapidly adapting esophageal mechanoreceptors in the mucosal and/or serosal layers, and LL-E responses might result from slowly adapting esophageal mechanoreceptors that were located in the muscular wall (Clerc and Mei 1983; Sengupta et al. 1990; 2006). In spinal visceral afferents innervating the esophagus in opossums, afferent fibers display rapid adaptation, and slow adaptation with or without after-discharges. Rapidly adapting fibers can become slowly adapting with higher intensities of esophageal distension (Sengupta et al. 1990). It has been

shown that STZ-induced diabetes increases after-discharges to mechanical stimulation in nociceptive primary afferent fibers innervating somatic receptive fields (Ahlgren et al. 1994). However, no consistent enhanced after-discharges in WDR neurons have been observed in STZ-induced diabetic rats (Pertovaara et al. 2001). In the present study, SL-E neurons were encountered more frequently in diabetic rats compared to control animals, which caused the mean duration of excitatory responses to noxious ED to be significantly shorter in spinal neurons recorded from diabetic rats than those in the control group. If the effect was at the esophageal receptor level, either a recruitment of rapidly adapting or a suppression of slowly adapting receptors might have occurred. Therefore, our data cannot exclude changes in visceroreceptive properties of spinal neurons in the presence of diabetic autonomic neuropathy partly resulting from peripheral visceral afferents and/or receptors in the esophagus.

LT and HT responses

In the present study, for spinal neurons excited by noxious ED in diabetic rats, 78.6% responded throughout the range of non-noxious and noxious ED and were classified as LT responses; this was higher than the percentage (56.1%) of LT responding neurons in the control group. The effect might be associated with behavioral allodynia during mechanical esophageal stimulation. Upregulation of spinal excitatory glutamate receptors and increased release of glutamate and substance P, as well as decreased activity in intraspinal inhibitory circuits may be important for the development of spinal neuronal hypersensitivity in diabetes (Kamei et al. 1990; Tanabe et al. 2005; Tomiyama et al. 2005; Wang et al. 2007). Interestingly, STZ-induced diabetes in our study significantly reduced excitatory responses and duration of responses of HT neurons to noxious ED, and also delayed excitatory responses of these neurons to noxious ED. In contrast, characteristics of LT responses to ED were not significantly different from those in the control group. The weaker responsiveness of HT neurons might be associated with damage of peripheral C afferent fibers originating from the esophagus during diabetes. In spinal (sympathetic) afferent fibers in opossum, two separate populations of esophageal mechanosensitive afferent fibers have been observed, including wide-dynamic-range or low threshold (66.7%) and high-threshold (Sengupta et al 1990). Of these, 60.5% fibers responding to ED are considered to be unmyelinated C fibers, and the remainders are myelinated A-delta fibers. Some studies have shown that diabetic mellitus damages small diameter C afferent fibers with respect to molecular, functional and morphometric measurements (Ahlgren and Levine 1994; Chen and Levine 2001; 2003; Kimura et al. 2005; Kamiya et al. 2005). The damage to C fibers in the peripheral nerve and small neurons in dorsal root ganglia results in greater wind-up discharges elicited by repetitive conditioning stimulus, increased frequency of denervated Schwann cells and regenerating fibers, collagen pockets, decreased expression of insulin receptor, nerve growth factor, substance P and CGRP (Kamiya et al. 2005). Furthermore, different subsets of C fibers might be impaired by diabetic mellitus to different degrees, and these subsets might play different roles in either painful or painless diabetic neuropathies. For example, only one-third of C-fibers in STZ-induced diabetic rats exhibit a marked hyper-responsiveness to mechanical stimuli; those same fibers also have a greater mean conduction velocity than the C fibers that do not demonstrate hyper-responsiveness (Chen and Levine 2001; 2003). Two-thirds of C-fibers in diabetic rats fire at the same mean frequency as C-fibers in control rats (Chen and Levine 2001; 2003). Therefore, hypothetically, increased excitability in LT neurons and decreased responsiveness in HT neurons observed in the present study might represent complex impairments in subsets of A-delta and C spinal fibers originating from the esophagus. However, esophageal primary afferent activity was not examined in the present study.

Viscerosomatic convergence

Clinically, visceral pain originating from the esophagus is often referred to midsternal and epigastric areas, to the back, and sometime to the suprasternal notch, neck and face (Pollard

and Bloomfield 1931). Esophageal and somatic afferent convergence onto the same spinal neurons may play an important role in esophagosomatic referred pain (Garrison et al. 1992; Euchner-Wamser et al. 1993; Qin et al. 2003). In the present study, 80% of neurons in the diabetic group received convergent inputs from cutaneous receptive field, which was not significantly different from 94% of neurons in the control group. The proportions of different classifications of spinal esophagosomatic convergent neurons in the diabetic group are similar to those in control group. Similar results were observed in a previous study, in which spinal dorsal horn neurons with somatic receptive fields were examined in STZ-induced diabetic rats (Pertovaara et al. 2000). In that study, increased responsiveness was observed in WDR neurons. It was further suggested that the effects were causally related to behavioral evidence of mechanical hypersensitivity in diabetic mellitus (Pertovaara et al. 2000). However, another study showed that, in normal rats, 87.5% of STT neurons were classified as HT neurons; only 9.3% STT neurons were classified as WDR neurons; whereas, in diabetic rats, the population of WDR neurons was 48.6% of STT neurons, while HT neurons was 35.1% of STT neurons. The remaining (16.2%) STT neurons were LT neurons (Chen and Pan 2002). Differences between these studies might be due to the different type of neurons examined for somatic inputs, since STT neurons are a specific group of spinal neurons with projections to the thalamus. Furthermore, in the present study, spinal neurons with large somatic receptive fields were encountered more frequently in diabetic rats than in control group. This observation agreed with findings in a previous study, in which the size of the receptive field of STT cells in STZ-induced diabetic rats is significantly larger than that of STT neurons recorded in normal animals (Chen and Pan, 2002). It also is consistent with clinical observations, in which the multi-modal and multi-segmental sensory testing approach is used to examine sensitivity to mechanical, thermal and electrical stimulations of the esophagus. Results show that esophageal hypersensitivity is accompanied by a 46% increase in the somatic referred pain area, indicating increased excitability of viscerosomatic convergent neurons in the spinal cord (Frokjaer et al. 2007; Arendt-Nielsen et al. 2000). However, the present results were different from another observation, in which the size of the receptive fields of WDR neurons characterized with a noxious mechanical stimulus is not significantly different between the diabetic and control groups (Pertovaara et al. 2000). It is possible that different subgroups of spinal neurons (viscerosomatic convergent or non-convergent) examined in these studies may be partially responsible for this difference.

Potential implications

Our electrophysiological study provided substantial evidence that there are functional changes in upper thoracic spinal neurons with esophageal mechanical inputs following STZ-induced diabetes in rats. The higher number of LT neurons and enlarged somatic receptive fields of esophagosomatic convergent neurons suggested the occurrence of spinal neuronal hypersensitivity in diabetic rats, whereas the weaker responsiveness of HT neurons and the shorter duration of excitatory responses of spinal neurons to noxious ED indicated neuronal hyposensitivity. Apparently, complex altered discharge characteristics in some subsets of upper thoracic spinal neurons, including spinal neuronal hyper- or hypo-sensitivity to mechanical esophageal stimulus, are observed in present model of diabetes. It is generally believed that current models of diabetes with STZ-treated rats develop mechanical, thermal, and chemogenic hyperalgesia and/or allodynia (Calcutt 2004). These sensory abnormalities are proposed to reflect the painful aspect of human diabetic neuropathy. In contrast, STZ-induced diabetic mice and C57Bl/6 mice display reduced behavioral responses to noxious stimuli, reflecting the insensate aspect of the human syndrome (Christianson et al. 2003; Chu et al. 2008; Johnson et al. 2007). Clinically, diabetes-induced nerve dysfunction can produce a variable degree of sensory and motor symptoms. Sensory loss develops in the majority of diabetic patients, including numbness and insensitivity to pain or touch. Painful symptoms (paresthesias, hyperalgesia, tactile allodynia) are only reported in one third or fewer patients

(Frokjaer et al. 2007; Sorensen et al. 2002). Early in the diabetic disease process hyperalgesia tends to be manifested, but over time, patients begin to experience hypoalgesia. An overall somatic and/or visceral hyperalgesia and/or allodynia in individual diabetic patients could alternately exist with hypoalgesia (Chu et al. 2008; Frokjaer et al. 2007). Most of the other studies focused on the somatic rather than visceral nociceptive sensitivity. Recently, a multi-modal esophageal testing approach applied to explore the mechanism underlying autonomic neuropathy in diabetic patients also indicated an impaired balance between peripheral hyposensitivity and central hypersensitivity mechanisms for esophageal reception (Frokjaer et al. 2007). In addition to abnormalities of peripheral afferent nerves, other central mechanisms, such as altered balance of descending inhibitory and facilitatory pathways, altered spinal intrinsic inhibitory circuits, or interaction between visceral and somatic afferents also may contribute to the development of diabetic esophageal neuropathy (Tanabe et al. 2005; Pertovaara et al. 2000). Further studies might include animal models for longer term evaluation to explore hyper- versus hypo-algesic properties of somatic and visceral diabetic neuropathies.

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Fig. 1.

Response patterns of thoracic spinal neurons to esophageal distension (ED, 0.4 ml, 20s). *A*: a neuron with short-lasting excitatory (SL-E) response. *B*: a neuron with long-lasting excitatory (LL-E) response. *C*: a neuron excited and inhibited (E-I) by ED. *D*: a neuron with inhibitory (I) response. *E*: comparison of response patterns of spinal neurons in diabetic and control groups. *F*: comparison of proportions of SL-E and LL-E neurons in diabetic and control groups. In panels A–D, the upper trace is the rate histogram and the lower trace is raw cell activity. The horizontal bars in this figure and the subsequent ones represent application of stimuli.

Fig. 2.

Low- and high-threshold responses of thoracic spinal neurons to graded ED. *A*: a spinal neuron with low-threshold excitatory (LT-E) response to ED. *B*: a spinal neuron with high-threshold excitatory (HT-E) response to ED. *C*: comparison of proportions of low and high threshold neurons responsive to ED in diabetic and control groups.

Fig. 3.

Comparison of locations of thoracic spinal neurons responding to ED in diabetic and control animals. *A*: lesion sites of spinal neurons responding to ED in diabetic animals. Circles (●) represent neurons excited by ED. Triangles (▲) represent neurons inhibited by ED. Squares (■) represent neurons excited/inhibited by ED. *B*: Lesion sites of spinal neurons responding to ED in control animals. *C*: spinal laminae of gray matter of T3 segment (drawing from Molander et al. 1989). I-X, laminae; Liss, Liss's tract; LSN, lateral spinal nucleus; Pyr, pyramidal tract; IM, intermedial nucleus. *D, E*: comparison of proportions of superficial and deeper neurons responding to ED in diabetic and control animals.

Fig. 4.

Characteristics of thoracic spinal neurons receiving somatic inputs. *A*: a neuron with low threshold (LT) response to brushing hair of skin. Right drawing shows location of somatic field. Br, brush, Pi, pinch. *B*: a wide dynamic range (WDR) neuron excited by both brush and pinch of somatic field. *C*: a neuron with high threshold (HT) response only to pinch of somatic field. *D*: Comparison of somatic field properties of spinal neurons responding to ED recorded from diabetic and control animals. *E*: comparison of somatic field sizes of spinal neurons that responded to ED in diabetic and control animals.

Comparison of characteristics of thoracic spinal neurons responding to noxious esophageal distensions (0.4 ml) in diabetic and control rats.

Table 1

Groups	Neuron classes	n	Spontaneous activity (imp/s)	Latency (s)	Excitatory responses (imp/s)	Inhibitory responses (imp/s)	Duration of responses (s)
Control	E	41	7.1±1.1	1.9±0.4	13.1±1.7	N/A	31.3±3.1
	I	6	6.5±1.8	1.3±0.4	N/A	4.0±0.9	29.2±5.5
	E-I	3	7.7±2.0	0.9±0.4	9.4±3.7	4.3±1.3	28.0±7.3
Diabetic	E	42	9.8±1.5	1.3±0.4	15.0±1.5	N/A	25.9±2.7*
	I	10	8.6±1.5	1.0±0.4	N/A	6.2±0.9	37.6±12.7
	E-I	3	7.8±2.5	0.6±0.1	18.9±9.0	5.9±1.3	53.0±3.8

E, excitatory responses. I, inhibitory responses. E-I, excitatory-inhibitory responses.

* P<0.05 compared to corresponding activity of neurons with excitatory responses in control group.

Table 2

Comparison of short- and long-lasting excitatory responses (SL-E, LL-E) of thoracic spinal neurons responding to noxious esophageal distension (0.4 ml) in diabetic and control rats.

Groups	Neuron classes	n	Spontaneous activity (imp/s)	Latency (s)	Excitatory responses (imp/s)	Duration of responses (s)
Control	SL-E	15	7.1±1.8	2.1±0.6	11.1±3.1	16.4±1.3
	LL-E	26	7.1±1.4	1.8±0.5	14.3±2.1	39.9±3.9
Diabetic	SL-E	27	11.0±1.6	1.0±0.4	14.7±1.9	18.6±0.7
	LL-E	15	7.7±2.1	2.0±0.6	15.5±1.6	39.1±5.1

Table 3
Comparison of low- and high-threshold excitatory responses (LT-E, HT-E) of thoracic spinal neurons responding to noxious esophageal distension (0.4 ml) in diabetic and control rats.

Groups	Neuron classes	n	Spontaneous activity (imp/s)	Latency (s)	Excitatory responses (imp/s)	Duration of responses (s)
Control	LT-E	23	9.5±1.4	1.1±0.3	12.6±1.8	31.4±3.5
	HT-E	18	4.0±1.3 [†]	2.9±0.7 [†]	13.9±3.3	31.2±5.5
Diabetic	LT-E	33	10.6±1.5	0.7±0.2	17.1±1.5	27.8±2.9
	HT-E	9	6.8±1.8	3.7±1.0 [†]	7.4±1.1 [*]	19.0±2.3 [*]

^{*} P<0.05 compared to corresponding activity of LT-E neurons in diabetic and HT-E neurons in control group.

[†] P<0.05 compared to corresponding activity in LT-E neurons.

Comparison of excitatory responses of superficial and deeper thoracic spinal neurons responding to noxious esophageal distension (0.4 ml) in diabetic and control rats.

Table 4

Groups	Neuron classes	n	Spontaneous activity (imp/s)	Latency (s)	Excitatory responses (imp/s)	Duration of responses (s)
Control	Superficial	16	7.2±1.8	2.2±0.6	13.5±2.3	26.9±3.4
	Deeper	25	7.0±1.4	1.7±0.5	12.9±2.5	34.1±4.5
Diabetic	Superficial	12	8.6±1.6	0.6±0.4	16.1±2.9	21.2±1.0
	Deeper	30	10.2±1.7	1.6±0.4	14.5±1.5	27.8±3.3*

* P<0.05 compared to corresponding activity of superficial neurons in diabetic rats.