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Characterization of upper thoracic spinal neurons receiving noxious cardiac and/or somatic inputs in diabetic rats

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ABSTRACT

The aim of the present study was to examine spinal processing of cardiac and somatic nociceptive input in rats with STZ-induced diabetes.

Type 1 diabetes was induced with streptozotocin (50 mg/kg) in 14 male Sprague–Dawley rats and citrate buffer was injected in 14 control rats. After 4–11 weeks, the rats were anesthetized with pentobarbital, ventilated and paralyzed. A laminectomy enabled extracellular recording of T_3 spinal cord neuronal activity. Intrapericardial administration of a mixture of algogenic chemicals (bradykinin, serotonin, prostaglandin E_2 (all at 10^{-5} M), and adenosine (10^{-3} M)) was applied to activate nociceptors of cardiac afferent nerve endings. Furthermore, somatic receptive properties were examined by applying innocuous (brush and light pressure) and noxious (pinch) cutaneous mechanical stimuli.

Diabetes-induced increases in spontaneous activity were observed in subsets of neurons exhibiting long-lasting excitatory responses to administration of the algogenic mixture.

Algogenic chemicals altered activity of a larger proportion of neurons from diabetic animals (73/111) than control animals (55/115, $P<0.05$). Some subtypes of neurons exhibiting long-lasting excitatory responses, elicited prolonged duration and others, had a shortened latency. Some neurons exhibiting short-lasting excitatory responses in diabetic animals elicited a shorter latency and some a decreased excitatory change. The size of the somatic receptive field was increased for cardiosomatic neurons from diabetic animals. Cutaneous somatic mechanical stimulation caused spinal neurons to respond with a mixture of hyper- and hypoexcitability.

In conclusion, diabetes induced changes in the spinal processing of cardiac input and these might contribute to cardiovascular autonomic neuropathy in patients with diabetes.

1. Introduction

Diabetic neuropathy is a common complication of diabetes mellitus that can affect autonomic, sensory, as well as motor nerves and significantly impact quality of life of these patients (Thomas and Tomlinson, 1993). Cardiovascular autonomic neuropathy (CAN) is found in patients with diabetes at a prevalence of 7–90% (Vinik et al., 2003) and involves cardiomotor and vasomotor efferents as well as cardiac sensory afferent fibers (Ahluwalia et al., 1995; Koistinen et al., 1996; Vinik et al., 2003; Thomas and Tomlinson, 1993). CAN in diabetic patients is associated with increased risk of developing cardiovascular disease and silent myocardial ischemia, a decreased heart rate variation, orthostatic hypotension, resting tachycardia, exercise intolerance, and these patients furthermore have a higher overall mortality (Vinik et al., 2003; Johnstone and Kinzfogl, 2005; Thomas and Tomlinson, 1993; Vinik and Ziegler, 2007). Silent myocardial ischemia is found more commonly in diabetic patients with coronary artery disease compared to the corresponding non-diabetic patients (Ditchburn et al., 2001; Schultz, 2003; Vinik et al., 2003), and a few studies show that this occurs even in the absence of autonomic neuropathy (Ahluwalia et al., 1995), suggesting that autonomic neuropathy is not the only factor affecting the cardiac nociception during diabetes. It seems likely that altered pain perception might also be related to a sensory disorder or alterations of the central integration of signals from the heart. Despite the huge impact altered pain perception potentially can have on the morbidity and mortality in diabetic patients, as yet, very little is known about cardiac sensory function and its central processing during diabetes. Just a few studies have shown that cardiac vagal afferent function and cardiosomatic reflexes are impaired in streptozotocin (STZ)-induced

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diabetic rats when compared to control animals (Gouty et al., 2001; Schultz, 2001; Liu et al., 2011).

A characteristic of neuropathy is decreased nerve conduction velocity, in humans as well as in animals, and has been shown to develop both centrally (Suzuki et al., 2000) and peripherally (Biessels et al., 1996; Kalichman et al., 1998; Nemeth et al., 1999; Walker et al., 1999; Nemeth et al., 2001; Selvarajah et al., 2006). In rats, nerve conduction velocity is decreased 4 weeks after the onset of type 1 diabetes (Biessels et al., 1996; Kalichman et al., 1998; Nemeth et al., 2001) and decreases with duration of diabetes (Nemeth et al., 1999). Animal experiments concerning sensory nerves during diabetes typically focus on painful diabetic neuropathy, a sensory neuropathy, which is a common cause of morbidity in diabetic patients. Behavioral studies using type 1 diabetic animals have shown the presence of this form of neuropathy, with a consensus on the presence of mechanical hyperalgesia and tactile allodynia in the paw, but conflicting results as to whether the thermal threshold is altered (Pertovaara et al., 2001; Chen and Pan, 2002; Khan et al., 2002; Morgado and Tavares, 2007). Studies on primary afferent somatosensory fibers show that the mechanical threshold is decreased for Aδ fibers and their responsiveness augmented during diabetes; whereas for C-fibers, the mechanical threshold is unchanged and the responsiveness ranges from no alteration to hyperresponsiveness (Ahlgren et al., 1992; Chen and Levine, 2001; Khan et al., 2002; Chen and Levine, 2003).

Changes have also been found in the spinal cord during diabetes. Selvarajah et al. found a decreased cross-sectional area of the 2nd and 3rd cervical segments of the spinal cord in diabetic patients with subclinical and clinical diabetic neuropathy (Selvarajah et al., 2006). Furthermore, chemogenic hypoalgesia was associated with a decrease in Fos expression in the dorsal horn of the spinal cord in STZ-diabetic C57BL/6 mice (Johnson et al., 2007), whereas mechanical hyperalgesia was found to be associated with an increase in Fos expression in STZ-diabetic rats (Morgado and Tavares, 2007). Furthermore, spinothalamic tract (STT) cells and spinal neurons from STZ-diabetic animals appeared to be hypersensitive following innocuous and noxious mechanical stimulation, and have higher neuronal spontaneous activity and enlarged somatic receptive fields (Pertovaara et al., 2001; Chen and Pan, 2002). However, the above studies are all based on somatosensory input. A recent electrophysiological study from our laboratory has investigated spinal neurons receiving visceral esophageal sensory afferent input in diabetic rats (Qin et al., 2009). In that study, altered activity of upper thoracic neurons receiving esophageal input was observed in STZ-diabetic rats. Tanabe et al. (2005) found changes in the intrinsic inhibitory system in the spinal cord that contributed to an increased spinal motor output in STZ-diabetic rats. Altered excitability of spinal neurons might be ascribed to functional changes of inhibitory descending pathways (Chen and Pan, 2002; Kimura et al., 2005; Tanabe et al., 2005), alterations in the intrinsic inhibitory system (Tanabe et al., 2005), sensitization of afferent fibers (Ahlgren et al., 1992; Chen and Levine, 2001; Khan et al., 2002; Chen and Levine, 2003), wind-up of C-fibers (Kimura et al., 2005), as well as receptor and neuropeptide changes (Kamei et al., 1990; Cloutier and Couture, 2000).

To the best of our knowledge, no prior studies have investigated the activity of upper thoracic spinal neurons receiving cardiac input in diabetic animals to determine if spinal neuronal processing is affected by this disease. Previously, our laboratory has performed electrophysiological investigations on upper thoracic T_3–T_4 spinal neurons in healthy rats after cutaneous somatic and chemical cardiac stimulation (Qin et al., 2002; Qin et al., 2003). Intrapericardial administration of inflammatory substances, known to be released during myocardial ischemia, changed activity of spinal neurons in the dorsal horn through activation of spinal cardiac sensory afferents in the heart. The majority of responsive neurons were excited and the remaining exhibited inhibitory or biphasic responses (Qin et al., 2003). Spinal neurons receiving cardiac input are modulated by descending – primarily inhibitory – input, interneurons and convergent somatic or visceral input (Qin et al., 2002; Qin et al., 2004b). The spinal neurons recorded are possibly projecting neurons, interneurons, and propriospinal neurons and therefore could be involved in intraspinal integration of nociceptive input, affecting both perception of anginal pain and autonomic cardiocardiac reflexes (Hobbs et al., 1992; Qin et al., 2001; Qin et al., 2002; Schultz, 2003). The purpose of the present study was to characterize the activity of upper thoracic (T_3) spinal neurons after noxious chemical stimulation of cardiac afferents and cutaneous somatic mechanical stimulation in rats with STZ-induced diabetes (type 1 diabetes). A preliminary report of parts of this work has been published in abstract form (Foreman et al., 2007).

2. Materials and methods

2.1. Induction of diabetes

A total of 28 Sprague Dawley rats (Harlan Inc., USA) were used in the present study. Type 1 diabetes was induced with a single injection of streptozotocin (STZ, 50 mg/kg in 0.1 M citrate buffer pH 4.5, i.p. in 14 rats at the age of 7 weeks). Citrate buffer was injected in the eight rats as an age- and weight-matched control group. An additional six animals were also used as controls. Two to three days later, the presence of diabetes was assessed by determining that blood glucose concentration was ≥20 mM in blood samples obtained from the tail using a strip-operated blood glucose sensor (One-Touch® Ultra®, LifeScan). Rats were weighed three times a week and blood glucose concentrations were measured once a week. All experimental protocols were approved by the Institutional Animal Care and Use Committee at The University of Oklahoma Health Sciences Center.

2.2. Surgical procedure

The surgical procedure has previously been described by Qin et al. (Qin et al., 2003). In short, 4–12 weeks after injection of STZ, rats were anesthetized with pentobarbital (60 mg/kg, i.p.). Sustained anesthesia was accomplished by continuous infusion of pentobarbital (15–25 mg/kg/h, i.v.) through a catheter in the left jugular vein. Mean arterial blood pressure was measured through a catheter in the right carotid artery. Artificial ventilation with a constant-volume pump (55–60 strokes/min, 3.0–5.0 ml stroke volume) was provided after tracheotomy. During spinal neuronal recordings, the animals were paralyzed with pancuronium bromide (0.4 mg/kg, i.v.) and supplementary doses were used to maintain muscle relaxation (0.2 mg/kg/h, i.p.). A thermostatically controlled heating pad and overhead infrared lamps kept rectal temperature between 36.7 °C and 37.3 °C throughout the experiment.

For chemical activation of the sensory nerve endings in the heart, a silicone tubing (0.020 cm i.d., 0.037 cm o.d., 14–16 cm in length) was passed through the thoracic thymus gland and inserted into the pericardial sac over the left ventricle.

To record extracellular action potentials rats were placed in the prone position and mounted in a stereotaxic headholder and stabilized with vertebral clamps at T_2 and T_6–T_8. A laminectomy was performed to expose the T_3 spinal segment. Then, dura mater and arachnoid were cut and the exposed spinal cord was covered with warm agar (3–4% in saline) for a stabile recording. Extracellular potentials of single T_3 spinal neurons were recorded with a carbon-filament glass microelectrode. The area of searching for neurons was approximately 0.5–2.0 mm lateral from midline of the spinal cord. Superficial neurons were recorded within 0.30 mm and deeper neurons within 0.31–1.20 mm from the dorsal surface of the spinal cord, corresponding, in rats, to lamina I–III and IV–VII + X, respectively (Molander et al., 1989; Ness and Gebhart, 1989).