Experimental Neurology 213 (2008) 439–447

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Effects of hyperglycemia on rat cavernous nerve axons: A functional and ultrastructural study

Elena G. Zotova [a,*], Herbert H. Schaumburg [a,b], Cedric S. Raine [a,b,c], Barbara Cannella [b], Moses Tar [d], Arnold Melman [d], Joseph C. Arezzo [a,c]

[a] Department of Neurology, Albert Einstein College of Medicine, Bronx, NY, USA
[b] Department of Pathology (Neuropathology), Albert Einstein College of Medicine, Bronx, NY, USA
[c] Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY, USA
[d] Department of Urology, Albert Einstein College of Medicine, Bronx, NY, USA

ARTICLE INFO

Article history:
Received 30 April 2008
Revised 9 July 2008
Accepted 11 July 2008
Available online 18 July 2008

Keywords:
Diabetes
Nerve conduction velocity
Small fiber neuropathy
Diabetic autonomic neuropathy
Erectile dysfunction
Neuroaxonal dystrophy
Cavernous nerve
Parasympathetic
Major pelvic ganglia
Cavernous smooth muscles

ABSTRACT

The present study explored parallel changes in the physiology and structure of myelinated (Aδ) and unmyelinated (C) small diameter axons in the cavernous nerve of rats associated with streptozotocin-induced hyperglycemia. Damage to these axons is thought to play a key role in diabetic autonomic neuropathy and erectile dysfunction, but their pathophysiology has been poorly studied. Velocities in slow conducting fibers were measured by applying multiple unit procedures; histopathology was evaluated with both light and electron microscopy. To our knowledge, these are the initial studies of slow nerve conduction velocities in the distal segments of the cavernous nerve. We report that hyperglycemia is associated with a substantial reduction in the amplitude of the slow conducting response, as well as a slowing of velocities within this very slow range (< 2.5m/s). Even with prolonged hyperglycemia (> 4months), histopathological abnormalities were mild and limited to the distal segments of the cavernous nerve. Structural findings included dystrophic changes in nerve terminals, abnormal accumulations of glycogen granules in unmyelinated and preterminal axons, and necrosis of scattered smooth muscle fibers. The onset of slowing of velocity in the distal cavernous nerve occurred subsequent to slowing in somatic nerves in the same rats. The functional changes in the cavernous nerve anticipated and exceeded the axonal degeneration detected by morphology. The physiologic techniques outlined in these studies are feasible in most electrophysiologic laboratories and could substantially enhance our sensitivity to the onset and progression of small fiber diabetic neuropathy.

Introduction

Diabetic autonomic neuropathy (DAN) is a critical and often life-threatening complication of diabetes that is generally poorly understood and inadequately studied (Vinik et al., 2003). DAN affects the cardiovascular, respiratory, gastrointestinal, genitourinary, thermoregulatory, papillary, and neuroendocrine systems (Boulton et al., 2005; Low et al., 2003; Vinik and Ziegler, 2007). The clinical onset and progression of DAN generally parallels the course of somatic neuropathy, but structural and functional deficits in autonomic nerves can occur in isolation or be dissociated from their somatic counterparts (Tentolouris et al., 2001; Töyry et al., 1997; Winkler et al., 2000; Young et al., 1986). Although DAN is usually considered a late stage complication of diabetes, sensitive measures can detect autonomic dysfunction at disease onset (Said, 2007). The incidence of DAN increases with age and duration of diabetes, thus this complication will likely gain increasing importance as the mean age of the population increases.

The role of DAN in cystopathy and erectile dysfunction (ED) is especially insidious (Sasaki et al., 2003). ED has an incidence of 35–75% in patients with diabetes (Vinik et al., 2003) and this complication is now considered a prediction tool for DM itself (Sun et al., 2006). ED may stem from deficits in the vascular system, penile smooth muscles and autonomic nerves (Hecht et al., 2001). The integrity of cavernous nerve (CN) is critical for normal erectile function; even minor surgical impairment of this nerve can lead to impotence (Klotz, 2004; Walsh and Donker, 2002). The CN is principally composed of unmyelinated axons with a few scattered myelinated fibers (Schaumburg et al., 2007). The myelinated fibers range in diameter from 1 to 8μm, with 2μm as the most common value; they originate in the dorsal root ganglia (DRG) or sympathetic chain and course through the pelvic or hypogastric nerves prior to entering the CN (Dail et al., 1989; Purinton et al., 1973). The majority of unmyelinated fibers are postganglionic parasympathetic and sympathetic axons originating in the major pelvic ganglia (MPG). A recent ultrastructural study in control rats

* Corresponding author. Fax: +1 718 430 8588.
E-mail address: zotova@aecom.yu.edu (E.G. Zotova).

0014-4886/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2008.07.009

confirmed the abundance of postganglionic cells in the proximal segment of the CN and described the scarcity of unmyelinated fibers in the distal third of the penis shaft (Schaumburg et al., 2007). In contrast, approximately 40% of the axons in the dorsal nerve of the penis (DNP), a sensory branch of the pudendal nerve, are myelinated (Hulsebosch and Coggeshall, 1982).

Functional and structural deficits in small diameter, myelinated (Aδ) and unmyelinated (C) axons are key components of DAN, ED and painful somatic neuropathies, but deterioration or improvement in activity of these axons is virtually undetected by standard nerve conduction velocity (NCV) procedures (Arezzo and Zotova, 2002). This limitation of the NCV technique is generally true for both clinical and pre-clinical studies and it diminishes the value of this important technique. The assessment of NCV in myelinated and unmyelinated small diameter fibers is a formidable task due to temporal dispersion, asynchronous signals, small amplitudes and phase cancellation (Arezzo and Zotova, 2002).

The present study examined parallel changes in the physiology and structure of small diameter axons in the CN of the streptozotocin-treated diabetic rat. Velocities in slow conducting fibers were measured by applying procedures originally designed to assess the relatively slow and often unorganized transmission of neural signals in the brain (Arezzo et al., 1986; Legatt et al., 1980; Steinschneider et al., 2008). Histopathology assessment examined step sections of the CN, MPG, and cavernous smooth muscle.

Materials and methods

Animals

Electrophysiology studies were performed on 10 Fischer rats (F344, Charles River Lab., Wilmington, MA); 7 were hyperglycemic and 3 were controls. An additional 8 Sprague–Dawley rats (SD, Harlan Sprague–Dawley, Inc., Indianapolis, Indiana) were used to optimize recording procedures and compare the NCV measure across strains. Histopathological studies were completed in two of the Fisher rats employed in the NCV studies, an additional 6 hyperglycemic Fischer rats and 5 age-matched controls. Histopathology was assessed at either 2, 4, 6, 8, or 10months following the onset of hyperglycemia (HG). HG was induced by a single 35mg/kg i.p. injection of streptozotocin (STZ). Prior to injection, STZ was dissolved in citrate buffer (60mL of 0.1M citric acid and 40mL of 0.2M Na2HPO4, pH 4.6). HG was confirmed by blood glucose levels above 300mg/dL. The mean blood glucose level in the STZ-treated rats was 394.1mg/dL (range 323 to 567mg/dL). All animals were given free access to standard laboratory chow and water throughout the study. CN electrophysiology is an invasive procedure so data were recorded at a single time point in each rat at a period ranging from 2 to 10months following the induction of HG. All procedures were approved by the Animal Institute Care and Use Committee at Albert Einstein College of Medicine.

Electrophysiological procedures

Data were collected from the CN, the DNP and the caudal nerve under isoflurane anesthesia (2.5–3.5%/O_2). For the CN, the lower part of the abdomen was opened by surgical incision and the ventral prostate was moved aside to expose the area around the MPG. The opening was kept narrow to diminish bleeding and drying or cooling of the exposed area. Rectal and near nerve temperature was monitored and maintained within 35–37°C using a circulating water heating unit. The main CN was located, and the recording electrodes were placed 2–3mm below its exit from MPG; the nerve was stimulated further distally at the base of the shaft of the penis (Fig. 1A).

Activity in the DNP was recorded antidromically using electrodes positioned along the proximal and distal portions of the penile shaft (Fig. 1A) and evoked by stimulation at the same site as employed for the CN. A small incision was made in the scrotum to expose the crura and the stimulating cathode was inserted into the corpus tissue to a depth of approximately 1–2mm at the base of the penis. At this location, both the CN and DNP run dorsal to urethra on their way to innervate corpus cavernosum (Walsh and Donker, 2002). Appropriate stimulation at this site can simultaneously trigger a response in both nerves. However, in some cases, the stimulating electrodes had to be moved distally along the penile shaft for optimal stimulation of the DNP, and to a point 2–3mm below the base of the penis for isolation of CN activity. Caudal nerve activity was measured on the dorsal surface of the tail 10mm below the hairline and 80mm proximal to the site of stimulation.

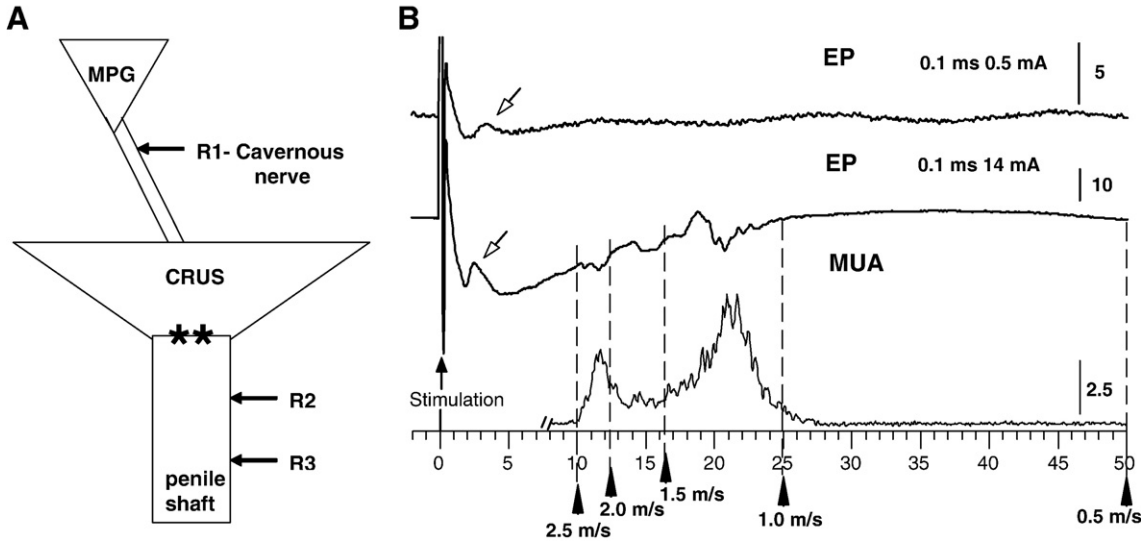

Fig. 1. Conditions of the recording of cavernous nerve activity. A. Schematic diagram illustrates the sites of recording and stimulation for the cavernous nerve and DNP. MPG-major pelvic ganglia, R1-recording site for cavernous nerve activity, R2-recording site for proximal DNP, R3-recording site used for calculation NCV of the distal segment of DNP between R2 and R3, ** — indicates position of stimulation. B. Responses of cavernous nerve are presented as evoked potentials (EP) and multi-unit activity (MUA) at two intensities of stimulation. Open arrows point out initial response of small diameter myelinated fibers (Aδ). Vertical dash lines separate the ranges of NCV. Calibration is given in microvolts, and time scale in milliseconds.