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BRAIN RESEARCH

Research Report

Voltage-dependent potassium currents of urethral afferent neurons in diabetes mellitus

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ABSTRACT

Urethra-to-bladder and urethra-to-urethra reflexes appear to be important for coordination of proper voiding. Diabetes mellitus (DM) is known to result in afferent neuropathy. Neuropathic alterations in electrophysiological properties of urethral afferent neurons may therefore contribute to voiding dysfunction seen in diabetes mellitus. Accordingly, we studied urethral afferent neuronal somata in streptozotocin-induced DM or age-matched vehicle controls by whole-cell patch clamp at 5- or 10-week time points. One week prior to study, Fast Blue was injected into the proximal urethra to label urethral afferent neurons. A previously undescribed diminution of afferent neuronal voltage-dependent potassium currents was a prominent feature of urethral afferent neuropathy in DM, acting to increase neuronal excitability. Thus, unlike bladder afferent neurons, urethral afferent neurons may be hyperexcitable well into DM progression.

1. Introduction

Patients with chronic diabetes mellitus (DM) suffer from lower urinary tract dysfunction which prominently includes impaired bladder sensation, increased bladder capacity, decreased bladder contractility, and increased post-voiding residual urine (Bradley, 1980; Ellenberg, 1980; Goldman and Appell, 1999; Mitsui et al., 1999). These changes are usually attributed to damage to both sensory and motor nerves serving the urinary bladder, within both the autonomic and somatic peripheral nervous system (Goldman and Appell, 1999; Mitsui et al., 1999; Nadelhaft and Vera, 1992; Steers et al., 1990). Several studies have shown that patients with DM have increased urethral outlet resistance (Kebapci et al., 2007; Menendez et al., 1996; Moller and Olesen, 1976) as well as cystopathic changes. Additionally, DM also damages the innervation of the urethra (Andersen and Bradley, 1976). While damage to the efferent innervation of the urethra may directly disrupt the contraction and relaxation of urethral smooth muscle (autonomic efferents) and external urethral sphincter (EUS) striated muscle (somatic efferents), damage to the afferent innervation of the urethra would be no less disruptive. Activation of urethral afferents not only affects urethral continence reflexes but also augments (Andersson, 2002; de Groat et al., 2001; Gustafson et al., 2003; Jung et al., 1999) or inhibits (Conte et al., 1989; Thor and Muhlhauser, 1999) reflex

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Abbreviations: DM, diabetes mellitus; EUS, external urethral sphincter; DRG, dorsal root ganglia; NGF, nerve growth factor; TRPV, transient receptor potential vanilloid receptor; STZ, streptozotocin

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bladder contraction. Thus, DM-induced urethral afferent neuropathy would compound the effects of the better-known bladder neuropathy (Daneshgari et al., 2006; Goldman and Appell, 1999; Longhurst et al., 2004; Steers et al., 1990; Steers et al., 1994).

Primary afferent neurons are affected by DM. At the extreme, DM is associated with apoptosis of primary afferent neurons in the dorsal root ganglia (DRG) (Guo et al., 2004; Schmeichel et al., 2003). Additionally, a variety of changes in membrane currents of primary afferent neurons has been recorded, including changes in sodium currents (Hirade et al., 1999; Hong et al., 2004; Hong and Wiley, 2006; Okuse et al., 1997; Shah et al., 2001) and calcium currents (Hall et al., 1995; Hall et al., 2001; Ristic et al., 1998; Umeda et al., 2006; Yusaf et al., 2001). Because membrane current changes may be important in the genesis of diabetic urethral neuropathy and thus lower urinary tract dysfunction in DM, we initiated whole-cell patch clamp study of the properties of urethral afferent neurons. As described in this paper, we found that a hitherto undescribed diminution of urethral afferent neuronal voltage-dependent potassium currents was a prominent feature of urethral afferent neuropathy in DM, acting to increase neuronal excitability.

2. Results

2.1. Blood glucose

Blood glucose levels were significantly higher in DM rats than in controls ($P<0.001$), but the difference between 5- and 10-week duration was not statistically significant ($P=0.59$) (Table 1).

2.2. Body weight change (Table 1)

Differences in body weight gain (weight at time of study — initial weight) between 5 and 10 weeks were significantly affected by treatment ($P=0.038$ for treatment × time interaction). Differences in weight gain between control and DM rats were statistically significant at 10 weeks ($P<0.001$) but not at 5 weeks ($P=0.068$).

2.3. Urethral afferent neuron size

In all, 77 neurons from 22 rats were studied: 10 from control rats studied at 5 weeks, 29 from control rats at 10 weeks, 18 from DM rats at 5 weeks, and 20 from DM rats at 10 weeks. (Seven neurons distributed across the 4 groups that were found to have resting potentials less negative than −40 mV were not studied.) Fig. 1 shows probability density plots for the diameters of the neurons patch clamped in this study from each group of animals. The distributions are similar except that fewer large diameter neurons were studied in DM animals, especially at 10 weeks. Because neurons cannot be considered to be truly randomly selected for study, this small difference cannot on its own be regarded as indicative of a real difference among the populations from which these neurons were selected.

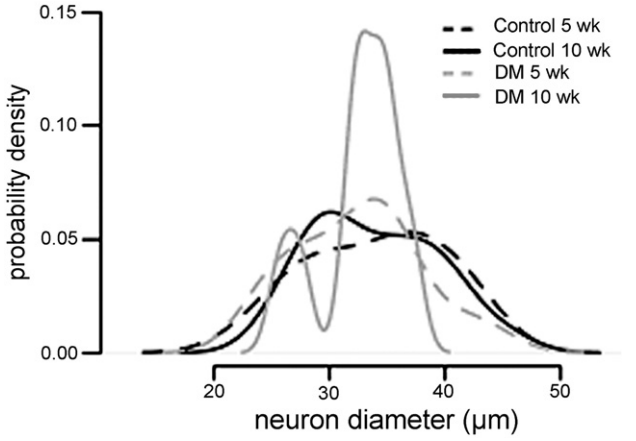

Fig. 1 – Neuronal size distribution at 5 and 10 weeks in control and DM animals. The distributions appear similar, except that fewer large diameter neurons were studied in DM animals, especially at 10 weeks. Mean neuron size was 34.0 (SD 6.0) μm in 5-week controls, 33.9 (SD 5.5) μm in 10-week controls, 32.4 (SD 5.5) μm in 5-week DM, and 32.6 (SD 3.5) μm in 10-week DM.

2.4. Resting potentials, action potentials, and threshold currents

The resting potential in DM rat neurons was depolarized relative to controls ($P=0.015$). Action potential overshoot and

Table 1 – Blood glucose levels and weight gain in the 4 groups of rats

	Duration (week)	Treatment	
		Control	DM
Blood glucose (mg/dl)	5	88±4	388±18
	10	97±3	407±5
Weight gain (g)	5	52±12	14±7
	10	92±10	−7±25

Values shown as mean±SEM. Numbers of rats: 5-week control 4, 10-week control 8, 5-week DM 6, and 10-week DM 4.

Table 2 – Membrane and action potential properties

	Duration (week)	Treatment		P_t	P_d
		Control	DM		
Resting potential (mV)	5	−61±2	−54±2	0.015	0.53
	10	−58±2	−54±2		
AP overshoot (mV)	5	55±4	51±4	0.28	0.053
	10	47±3	43±2		
After hyperpolarization (mV)	5	−9±2	−13±1	0.12	0.054
	10	−10±1	−8±1		
AP duration (ms)	5	2.6±0.6	5.4±1.4	0.026	0.55
	10	4.0±0.8	4.3±0.6		
Threshold potential (mV)	5	−36±2	−28±2	0.040	0.94
	10	−33±2	−30±3		
Threshold current (nA)	5	4.2±0.8	3.4±0.3	0.066	0.15
	10	3.8±0.3	2.8±0.2		

Data shown as mean±SEM. Numbers of neurons: 5-week control 10, 10-week control 29, 5-week DM 18, and 10-week DM 20.
AP = action potential, P_t = P-value for treatment (control vs. DM), P_d = P-value for treatment duration (5 vs. 10 weeks).