63

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HYPEREXCITABILITY OF CAPSAICIN SENSITIVE BLADDER AFFERENT NEURONS FROM MICE WITH NEUROGENIC DETRUSOR OVERACTIVITY INDUCED BY SPINAL CORD INJURY

Hypothesis/aims of study

Spinal cord injury (SCI) rostral to the lumbosacral level eliminates voluntary and supraspinal control of voiding, leading initially to areflexic bladder and urinary retention, and then to a slow development of spontaneous voiding and neurogenic detrusor overactivity (NDO) mediated by spinal micturition reflex pathways [1]. In chronic SCI rats, unmyelinated C-fibers in bladder afferent pathways show increased excitability [2] and are responsible for initiating NDO, because desensitization of C-fiber afferents by systemic capsaicin administration eliminates nonvoiding contractions (NVCs) [1]. However, a relationship between SCI-induced changes in properties of C-fiber bladder afferent pathways and the SCI-induced NDO has not been well characterized in mice when compared to rats. In this study, we therefore examined; (1) the contribution of capsaicin-sensitive C-fiber afferent pathways to NDO as shown by NVCs during cystometry and (2) changes in excitability of capsaicin sensitive bladder afferent neurons from SCI mice focusing on action potentials and voltage-gated K^+ (Kv) currents, which are major determinants of neuronal excitability.

Study design, materials and methods

In female C57BL/6 mice (9-10 weeks old), the spinal cord was transected at Th8-Th9 level. 1. Cystometry: At 4 weeks after SCI, cystometory was performed under an awake condition in SCI mice without or with capsaicin pretreatment (50 mg/kg, sc) that was performed 4 days prior to cystometry.

2. Patch clamp study: Bladder afferent neurons were labelled with axonal transport of Fast Blue (FB), a fluorescent retrograde tracer, injected into the bladder wall 3 weeks after SCI. Four weeks after SCI, L6-S1 dorsal root ganglion (DRG) neurons were prepared and whole cell patch clamp recordings were performed on FB positive neurons (=bladder afferent neurons). After recording of action potentials or Kv currents, capsaicin sensitive neurons were identified by a transient inward current evoked by capsaicin.

Two major types of Kv currents expressed in C-fiber DRG neurons, namely slow decaying A-type K^+ (slow K_A) and sustained delayed rectifier-type K^+ (sustained K_{DR}) currents were evaluated [3]. In these neurons, slow K_A currents are activated by depolarizing voltage steps from hyperpolarized membrane potentials and inactivated when the membrane potential is maintained at a depolarized level more than -40 mV [3], therefore, slow K_A currents are estimated by the difference in these currents activated by depolarizing voltage pulses from a holding potential (HP) of -40 mV and from a HP of -120 mV (see Fig. 2).

Results

1. Capsaicin pretreatment significantly (P<0.05) reduced the number of NVCs in SCI mice compared to untreated SCI rats.

2. The resting membrane potentials and the peak and duration of action potentials in capsaicin sensitive bladder afferent neurons did not differ between SCI and control spinal intact (SI) mice (Table 1). On the other hand, the threshold for eliciting action potentials in neurons from SCI mice was significantly lower than the measurement in neurons from SI mice (Fig. 1 and Table 1). Also, the number of action potentials during 800-milisecond membrane depolarization in capsaicin sensitive bladder afferent neurons from SCI mice was significantly greater than that in SI mouse neurons (Fig. 1 and Table 1). In addition, the diameter and cell input capacitance of capsaicin sensitive bladder afferent neurons from SCI mice were significantly greater than those of SI mouse neurons (Table 1). Densities of slow K_A and sustained K_{DR} currents evoked by depolarization to 0 mV in capsaicin sensitive bladder afferent neurons from SCI mice were significantly lower than those measured in SI mouse neurons (Fig. 2 and Table 1).

Interpretation of results

Our results indicate that; (1) SCI-induced NDO is dependent at least in part on C-fiber afferent activation, (2) capsaicin sensitive bladder afferent neurons from SCI mice show hyperexcitability as evidenced by lower spike activation thresholds and tonic firing pattern, and (3) Kv current densities, both slow K_A and sustained K_{DR}, are reduced in these neurons from SCI mice. Therefore, functional changes in Kv channels could be responsible for the hyperexcitability of bladder afferent neurons in SCI mice. In addition, SCI induced somal hypertrophy of these neurons in mice as evidenced by greater cell diameter and input capacitance, which was proportional to membrane surface area. Taken together, morphological and functional changes in capsaicin sensitive C-fiber bladder afferent neurons might be involved in enhanced synaptic transmission in the spinal cord, leading NDO in SCI mice.

Concluding message

SCI-induced reduction in both slow K_A and sustained K_{DR} current densities contributes to the emergence of NDO as well as hyperexcitability of capsaicin sensitive C-fiber bladder afferent neurons in mice.

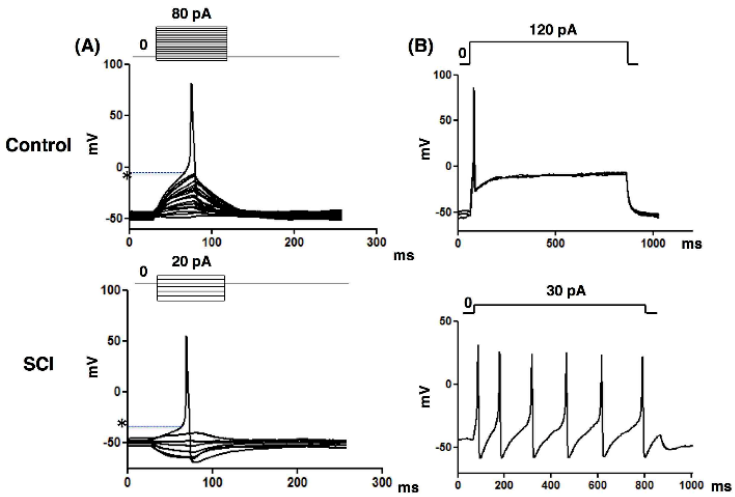

Fig. 1. Representative recordings of action potentials (A) and firing patterns (B) in capsaicin sensitive bladder afferent neurons from control and SCI mice. Asterisks with dash lines indicate the thresholds for spike activation.

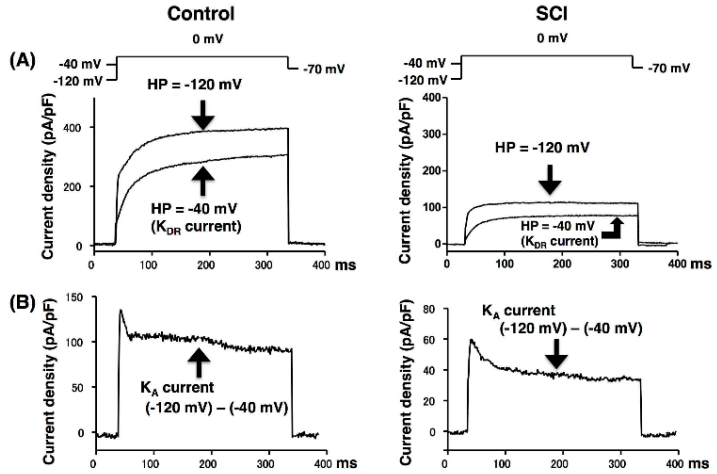

Fig. 2. Changes of K_V currents in capsaicin sensitive bladder afferent neurons from control and SCI mice. (A) Representative recordings show superimposed outward K^+ currents evoked by voltage steps to 0 mV from -120 and -40 mV holding potentials (HP). (B) K_A currents are obtained by subtracting K^+ currents evoked by depolarization to 0 mV from -40 and -120 mV HP.

Table 1. Electrophysiological properties of capsaicin sensitive bladder afferent neurons from mice

	Control	SCI
Spikes:		
Number of cells/mice	13/7	16/4
Diameter (μm)	25.9 ± 1.2	29.9 ± 0.7*
Input capacitance (pF)	24.2 ± 2.4	36.4 ± 2.7*
Resting membrane potential (mV)	-49.1 ± 1.3	-50.0 ± 0.1
Spike threshold (mV)	-25.4 ± 1.2	-31.4 ± 1.4*
Peak membrane potential (mV)	38.1 ± 2.9	35.5 ± 3.4
Spike duration (ms)	3.5 ± 2.9	2.9 ± 0.2
Number of spikes (800 ms depolarization)	2.3 ± 0.7	4.6 ± 0.7#
Density:		
Number of cells/mice	22/7	23/8
K_A current density (pA/pF)	45.2 ± 7.8	23.5 ± 3.2*
K_{DR} current density (pA/pF)	116.9 ± 18.0	52.6 ± 6.3*

K^+ current densities are normalized with respect to cell input capacitance. Values present as means ± S.E.M. *P<0.05, vs control by Student t-test. #P<0.05, vs control by Mann-Whitney U test.

References

1. de Groat WC, Yoshimura N. Mechanisms underlying the recovery of lower urinary tract function following spinal cord injury. Prog Brain Res. 2006;152:59-84.
2. Yoshimura N, de Groat WC. Plasticity of Na+ channels in afferent neurons innervating rat urinary bladder following spinal cord injury. J Physiol. 1997;503:269-276.
3. Yoshimura N, de Groat WC. Increased excitability of afferent neurons innervating rat urinary bladder after chronic bladder inflammation. J Neurosci. 1999;19:4644-4653.

Disclosures

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