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Research Report

Transient suppression of the vesicular acetylcholine transporter in urinary bladder pathways following spinal cord injury

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ABSTRACT

The aim of this study was to examine the expression profile of the vesicular acetylcholine transporter (VAChT), which is a cholinergic pre-synaptic marker, in the lower neural tract following spinal cord injury (SCI) and its effect on coordination of micturition. In adult female Sprague–Dawley rats, SCI was induced by complete transection of the spinal cord at T9. At various time points, 3, 7, 14 and 28 days, after SCI, cystometry was performed on conscious rats. Bladder areflexia was observed during the first week. Twenty-eight days after SCI the rats showed reflex contractions and voiding. The expression of VAChT was examined with immunohistochemistry. The number of VAChT-positive nerve terminals, which were surrounding neuronal soma, was transiently decreased in pelvic ganglion and spinal cord (L1, L2, L6 and S1). In particular VAChT terminals surrounding motor neurons in the ventral horn and autonomic pre-ganglion cells were dramatically decreased from 3 to 14 days after SCI. Similarly, and the number of VAChT-positive fibers in the bladder wall was also decreased. The intensity of VAChT terminals recovered in all above regions in conjunction with recovery of bladder function. These observations indicate that the transient decrease of the VAChT-positive nerve might cause a failure of cholinergic neuronal transmission along the urinary bladder tract after SCI. As the cholinergic system was recovered at least in rat, the functional recovery of neurogenic bladder syndrome in SCI patients may become possible by further understanding the mechanism underlying the recovery of cholinergic system in rat.

1. Introduction

Spinal cord injury (SCI) above the lumbosacral level causes a phase of spinal shock and impairs voluntary micturition (de Groat, 1995; de Groat et al., 1990; Kruse et al., 1993). Concomitantly, it evokes inhibition of sphincter activity resulting in a hyperreflexia of the bladder and the external sphincter (detrusor–sphincter–dyssynergia) (Kruse et al., 1994; Seki et al., 2002; Yoshiyama et al., 1999). Also SCI induces functional bladder outlet obstruction, and increasing

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Abbreviations: BSA, bovine serum albumin; ChAT, choline acetyl transferase; FDI, fiber density index; IR, immunoreactivity; PBS, phosphate buffer saline; Per, peripherin; SCI, spinal cord injury; VAChT, vesicular acetylcholine transporter

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micturition pressure and detrusor muscle hypertrophy (Keast, 1999; Kruse et al., 1995; Yoshiyama et al., 1999). And it is known that rat and human exhibit similar micturition dysfunction after SCI (Kruse et al., 1993). However, in rat the bladder reflex activity slowly recovers within a few weeks following SCI and initiates voluntary micturition in chronic paraplegic animals (Chancellor et al., 1994). Thus, understanding the molecular mechanisms implicated in the hypofunction and subsequent amelioration after SCI would provide a basis for enhancing reorganization of lower urinary tract function after SCI in humans.

Micturition consists of two main functions, storage and voiding, and the voluntary control of voiding is regulated by a complex mechanism in the spinal and supraspinal neural pathways (Hoang et al., 2006). In rats, voiding is normally mediated by contraction of the detrusor accompanied by coordinated activation of the external sphincter (de Groat, 1995; de Groat et al., 1990; Pikov and Wrathall, 2001). Motor neurons activating the external sphincter are located in the dorsolateral nucleus of the L6–S1 ventral horn (Pikov and Wrathall, 2001; Schroder, 1980), and those activating the bladder are part of sacral parasympathetic nuclei (Nadelhaft and Booth, 1984; Pikov and Wrathall, 2001), receive direct or indirect supraspinal projections mainly from Barrington's nucleus (Marson, 1997; Nadelhaft and Vera, 1996; Vizzard et al., 1995) in the brain stem. Efferent pathways from the thoracolumbar (T12–L2) and lumbosacral (L5–S1) levels of the spinal cord project to the lower urinary tract via nerves as follows: (1) the hypogastric nerve, which carries sympathetic pre-ganglionic inputs from lumbar cord, or (2) pelvic nerve, which carries parasympathetic inputs from sacral cord; the and (3) pudendal nerve, whose motor neurons originate in the ventral horn of segments L6–S1 (Callsen-Cencic and Mense, 1999; Marson and Gravitt, 2004; McKenna and Nadelhaft, 1986). Accordingly, orchestration of those systems could be crucial for micturition.

In order to evaluate activities of those efferent pathways morphologically, identification of cholinergic inputs would be useful because those systems use acetylcholine as a major neurotransmitter (Schafer et al., 1994). To examine the alteration of cholinergic synaptic inputs, we focused on the vesicular acetylcholine transporter (VAChT) as a marker for the cholinergic pre-synapse area. VAChT is a vesicle membrane protein and is responsible for the uptake of acetylcholine into synaptic vesicles (Erickson et al., 1996; Ferguson et al., 2003; Maeda et al., 2004). Both expression of VAChT mRNA and protein was demonstrated in a variety of cholinergic neurons, including peripheral motor and autonomic nerves (Arvidsson et al., 1997; Schafer et al., 1998). The most important feature of VAChT is its fairly restricted localization on cholinergic synaptic vesicles, and therefore every cholinergic terminal including autonomic and motor termini can be clearly visualized by immunohistochemistry using anti-VAChT antibody (Maeda et al., 2004; Schafer et al., 1998; Weihe et al., 1996). Furthermore, it was demonstrated that after cranial motor nerve injury, the protein levels of VAChT in endplate transiently disappeared, and along with the nerve regeneration the VAChT immunoreactivity recovered in the regenerated pre-synaptic terminal (Maeda et al., 2004). This suggested that the recovery of VAChT immunoreactivity in pre-synaptic terminals corresponded to the functional recovery. Therefore, here we have examined the alterations of VAChT expression in synaptic terminals of the urinary bladder pathway including spinal cord, pelvic ganglion and bladder after SCI.

2. Results

2.1. *Cystometry*

During the awake-cystometry, an infusion of saline into the bladder induced rhythmic bladder reflex contractions in normal rats, and bladder pressure during the infusion rapidly increased without detectable contractile activity until the initiation of reflex voiding. The amplitude (26.1±2.8 cm H_2O), duration (22.4±3.0 s) of bladder contractions and voided volume (0.4±0.1 ml) were similar to the measurements among normal rats (Fig. 1A). Voiding was totally abolished during the first week after SCI. At 3 days after SCI, bladder contractions were not observed at all (Fig. 1B). Then irregular bladder contractions started to occur at 7 days, though this voiding was not efficient enough to empty the bladder (Fig. 1C). From this period, the automatic micturition gradually returned. The activity of distended and hypertrophied bladder was clearly increased at 14 days post-operation (Fig. 1D). At 28 days, the high amplitude (40.7±3.4 mm H_2O) and long duration (35.3±6.0 s) following small amplitude (5.1±2.4 mm H_2O) contractions appeared and the voided volume (4.9±1.9 ml) increased (Fig. 1E). The bladders of SCI rats were notably distended resulting in increased voided volume, but the residual urine was still present 28 days following SCI.

2.2. *Immunohistochemistry*

2.2.1. *VAChT-IR in bladder*

The bladders of SCI rats were distended and the bladder walls markedly thickened compared with that of normal rats at the time of bladder removal. In normal rats, VAChT-IR nerve fibers with varicosities were present along the detrusor bundle (Fig. 2A), and some minor populations of positive fibers were also observed in the lamina propria. To examine whether VAChT-IR structures corresponded to nerve, we have attempted the simultaneous labeling with the antibody against the pan-PNS nerve marker, peripherin (Per). All VAChT-positive fibers were simultaneously stained with peripherin immunoreactivity (Per-IR), although some Per-IR-positive fibers were negative to VAChT-IR suggesting that VAChT-IR-negative fibers would be sensory or sympathetic nerves. Initially we have examined both base and body parts of the bladder, and the fiber intensity was significantly higher in base part than in body. However, the alterations of the density of VAChT-IR fibers in the both regions after SCI demonstrated similar tendencies. Therefore, we presented the cases observed in base part of the bladder in this paper. In SCI animals VAChT-IR nerve fibers in the bladder walls were significantly decreased at 3 days after SCI (Fig. 2B), and further decreased at 7 days after SCI (the fiber density index: FDI 0.6±0.6, Figs. 2C and 3) compared with normal rats (FDI: 7.8±2.8, $p<0.001$). All the VAChT-IR fibers remained were