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Research Paper

Impairment of sensory afferents by intrathecal administration of botulinum toxin A improves neurogenic detrusor overactivity in chronic spinal cord injured rats

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ABSTRACT

Spinal cord injury (SCI) often leads to neurogenic detrusor overactivity (NDO) due to sprouting of sensory afferents on the lumbosacral spinal cord. NDO is characterized by high frequency of voiding contractions and increased intravesical pressure that may lead to urinary incontinence. The latter has been described as one of the consequences of SCI that mostly decreases quality of life. Bladder wall injections of botulinum toxin A (Onabot/A) are an effective option to manage NDO. The toxin strongly impairs parasympathetic and sensory fibres coursing the bladder wall. However the robust parasympathetic inhibition may inhibit voiding contractions and cause urinary retention in patients that retain voluntary voiding. Here, we hypothesised that by restricting the toxin activity to sensory fibres we can improve NDO without impairing voiding contractions.

In the present work, we assessed the effect of Onabot/A on sensory neurons in chronic (4 weeks) SCI rats by injecting the toxin intrathecally (IT), at lumbosacral spinal cord level. This route of administration was shown before to have an effect on bladder pain and contractility in an animal model of bladder inflammation. We found that IT Onabot/A led to a significant reduction in the frequency of expulsive contractions and a normalization of bladder basal pressure while maintaining voiding contractions of normal amplitude. Cleavage of SNAP-25 protein occurred mainly at the dorsal horn regions where most of the bladder afferents end. Cleaved SNAP-25 was not detected in motor or preganglionic parasympathetic neurons. A significant decrease in CGRP expression, a peptide exclusively present in sensory fibres in the spinal cord, occurred at the L5/L6 segments and associated dorsal root ganglia (DRG) after Onabot/A injection in SCI animals. Onabot/A strongly increased the expression of ATF3, a marker of neuronal stress, in L5/L6 DRG neurons.

Taken together, our results suggest that IT Onabot/A has a predominant effect on bladder sensory fibres, and that such effect is enough to control NDO following chronic SCI. The mechanism of action of Onabot/A includes not only the cleavage of SNAP-25 in sensory terminals but also impairment of basic cellular machinery in the cell body of sensory neurons.

1. Introduction

The incidence and prevalence of spinal cord injury (SCI) are difficult to estimate on a yearly basis but it is assumed that >2 million people worldwide live with SCI (Lee et al., 2014). Despite recent advances, SCI is still a highly debilitating condition. Depending on the magnitude of SCI, affected individuals may present complete or incomplete loss of sensory and motor functions. With adequate treatment, certain neurological problems may be overcome, at least partially, during the first year after SCI, and patients with incomplete cord sectioning recover some limb function (French et al., 2010). A critical aspect for SCI patients is loss of voluntary control over bladder function. As a matter of fact, urinary incontinence, due to development of neurogenic detrusor overactivity (NDO), is reported by most SCI patients and represents the major cause for a decreased quality of life (Simpson et al., 2012). Indeed, after improving motor and sensory function, regaining bladder control is the highest

Abbreviations: ATF3, activating transcription factor 3; CGRP, calcitonin gene-related peptide; cSNAP-25, cleaved SNAP-25; DRG, dorsal root ganglia; IT, intrathecal; NDO, neurogenic detrusor overactivity; Onabot/A, OnabotulinumtoxinA; SCI, spinal cord injury.

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priority for SCI patients (Collinger et al., 2013; French et al., 2010; Simpson et al., 2012), due to its heavy impact on social engagement and performance of daily tasks.

Experimental studies have shown that bladder C-fibres play a fundamental role in the appearance of NDO. De Groat and co-workers demonstrated that after chronic SCI in cats, NDO emergence could be abolished by the administration of systemic capsaicin (Cheng et al., 1995, 1999). These data suggested that after SCI, the synaptic reorganization responsible for the new abnormal spinal micturition reflex is mediated by C-fibres (de Groat et al., 1990). In chronic SCI rats, the sprouting of peptidergic fibres around the preganglionic parasympathetic neurons of the lumbosacral spinal cord confirmed the role of C-fibres in the previous physiological observations (Zhang et al., 2008). Accordingly, the impairment of C-fibres has been attempted for long time to control NDO. Intravesical administration of capsaicin and resiniferatoxin (RTX) has been assessed in pilot clinical trials with considerable success but unfortunately, were not followed by appropriate regulatory trials (Cruz et al., 1997; Fowler et al., 1994).

Current management of NDO include bladder injections of OnabotulinumtoxinA (Onabot/A) (Cruz et al., 2011; Kennelly et al., 2015; Schurch et al., 2000). Successful results rely on the toxin's ability to block neuroexocytosis, by cleaving the SNAP-25 protein required for the accurate assembly of synaptic vesicles with the pre-synaptic cell membrane (Chancellor et al., 2008). Thus, the detection of cleaved SNAP-25 (cSNAP-25) has been used as a reliable marker of Onabot/A activity both in peripheral and central nervous systems (Coelho et al., 2012a, 2012b, 2014; Oliveira et al., 2015). Onabot/A is a non-specific neurotoxin that impairs almost all motor but only half of sensory fibres coursing the bladder wall (Coelho et al., 2010, 2012a, 2012b). This dual effect may certainly contribute to the high cure rates of urinary incontinence and strong reduction in the maximal detrusor pressure obtained with this toxin in patients with NDO of spinal origin. However, in patients who void spontaneously the predominant action on parasympathetic fibres is also responsible for a high rate of urinary retention, forcing patients to initiate intermittent self-catheterization. (Cruz et al., 2011; Cruz and Nitti, 2014; Ginsberg et al., 2013).

It is accepted that an important step ahead in botulinum toxin field would be the development of a toxin that specifically targets sensory fibres. In this way one expects to control NDO while minimizing the risk of urinary retention as observed in the pilot studies with capsaicin or RTX. However, since modified toxins are not yet available, testing such hypothesis for botulinum toxin A requires a method of administration that only targets sensory fibres. In a recent study, we demonstrated a reduction in abdominal pain and bladder hyperactivity in a model of chronic bladder inflammation after intrathecal (IT) administration of botulinum toxin at L5/L6 spinal cord level (Coelho et al., 2014a, 2014b). Cleaved SNAP-25 was concentrated between L4 and L6 spinal cord segments and none could be seen on motor neurons or in preganglionic parasympathetic neurons of the intermediolateral gray matter. Accordingly, no locomotion deficits were observed in the injected animals and the ability of the bladder to contract and empty completely at voiding remained intact. Thus, in the present study we evaluated the predominant sensory effect of intrathecal administration of botulinum toxin on the bladder function of chronic SCI rats and investigate putative underlying mechanisms of action.

2. Methods

2.1. Animals

All experiments were performed in female Wistar rats (Charles River, France) weighing 220–250 g that were maintained under a 12 h light/12 h dark schedule, with free access to food and water. Experimental procedures were carried out according to the European Communities Council Directive 2010/63/EU. All efforts were done to reduce the number of animals used and their suffering.

2.2. Reagents and drugs

Surgeries were performed under deep anaesthesia induced by an intraperitoneal (i.p.) injection of a mixture of ketamine (6 mg/100 g of body weight) and medetomidine (0.025 mg/100 g of body weight) diluted in saline. For cystometries and terminal handling animals received a subcutaneous (s.c.) injection of urethane (1.2 g/kg of body weight). OnabotulinumtoxinA (Onabot/A) was purchased from Allergan, CA, USA. The rabbit polyclonal cSNAP-25 antibody raised against the truncated C-terminal peptide of SNAP-25, was a kind gift from Ornella Rossetto and has been previously described (Antonucci et al., 2008; Coelho et al., 2014a, 2014b). Mouse monoclonal calcitonin gene-related peptide [(CGRP) (ab81887)], rabbit polyclonal β-actin (ab8227) and mouse monoclonal glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (ab8245) antibodies were obtained from Abcam, Cambridge, UK. Rabbit polyclonal activating transcription factor 3 (ATF3) (SC-188) and HRP-labelled secondary antibodies were purchased from Santa Cruz Biotechnology, Germany. Alexa™ 568 and 488 fluorochrome-labelled secondary antibodies, produced in donkey, were obtained from Molecular Probes Europe, Life Technologies, UK.

2.3. Spinal cord transection and catheter implantation

The model of chronic SCI induced by a complete spinal cord transection at the level of low thoracic spinal segments T8/T9 was chosen for the present study (Cruz et al., 2006; de Groat et al., 1990; Frias et al., 2015). Animals were divided into four experimental groups: (A) spinal intact + saline IT (n = 8); (B) spinal intact + Onabot/A IT (n = 8); (C) SCI + saline IT (n = 10); (D) SCI + Onabot/A IT (n = 10). Groups A and B were used as sham-operated controls, in which the spinal cord was exposed but not sectioned. Groups C and D were submitted to SCI. All animals underwent surgical implantation of a sterile silicone catheter (SF Medical; internal diameter: 0.3 mm, outer diameter: 0.635 mm) (Coelho et al., 2014a, 2014b). Briefly, under deep anaesthesia animals were submitted to laminectomy at T8/T9 level. The meninges were pierced and a silicone catheter was inserted in the subarachnoid space and pushed until the tip reached the L5/L6 spinal cord segment. The other tip was sealed and placed subcutaneously at the back of the neck for drug delivery 4 weeks after surgery (saline for groups A and C or Onabot/A for groups B and D). Catheter placement was followed by complete sectioning of the spinal cord in groups C and D and haemostatic sponge was placed between the retreated ends of the cord. Animals were left to recover and carefully monitored, receiving a daily i.p. injection of antibiotic (ciprofloxacin, 1 mg/kg) for 10 days after surgery. Bladders were manually emptied by abdominal compression to avoid urinary retention, twice daily for a period of 2 weeks. Female rats were preferred due of the difficulty to manage urinary retention in male rats.

2.4. IT drug delivery and cystometries

Four weeks after surgery, the catheter tip at the animal back was externalized and saline or Onabot/A (5 U diluted in 50 μL of saline) were delivered. Three days later, animals underwent cystometry. Animals were anaesthetized (urethane 1.2 g/kg, subcutaneous injection) and body temperature was maintained at 37 °C with a heating pad. Bladders were exposed via a midline low abdominal laparotomy. A 21-gauge needle connected to an infusion pump and to a pressure transducer was inserted into the bladder dome. Animals were left untouched for 15–30 min to allow bladder stabilization, after which saline infusion was initiated (constant rate of 6 mL/h), while intraluminal pressure was measure and recorded for 1 h. The urethra remained