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Intrathecal delivery of resiniferatoxin (RTX) reduces detrusor overactivity and spinal expression of TRPV1 in spinal cord injured animals

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ABSTRACT

Recently, it has been demonstrated that intrathecal delivery of resiniferatoxin (RTX) produces strong analgesia, even in models of bone cancer pain. RTX has been investigated to treat bladder dysfunction of spinal origin, applied by intravesical instillation. However, RTX delivered by this route was not completely satisfactory in controlling urinary incontinence and high intravesical pressure. Thus, the present study assessed the effects of intrathecal injections of RTX in bladder dysfunction in rats with spinal cord transection (SCT).

Bladder function was evaluated in SCT rats 24 h following intrathecal administration of RTX. Detrusor overactivity and intravesical pressure were reduced in a dose-dependent manner. This was accompanied by a decrease in spinal cord TRPV1 and CGRP, but not in IB4 binding sensory fibres. Also, intrathecal RTX induced a dose-dependent reduction in spinal cord activation of the ERK pathway.

Overall, our results show that intrathecal administration of RTX effectively reduces detrusor overactivity and reduces intravesical pressure in models of complete chronic spinal cord transection by suppressing the activity of TRPV1 expressing afferent fibres. Also, intrathecal RTX decreases sensory input, as shown by reduced spinal ERK activation. These findings might be relevant for the management of patients with spinal cord injuries.

Introduction

After a period of detrusor arreflexia, spinal cord injuries (SCI) usually cause severe detrusor overactivity, a leading mechanism for urinary incontinence in SCI patients. In addition, detrusor overactivity may be associated with detrusor-sphincter-dyssynergia (DSD) (for review see Vizzard, 2006). This loss of coordination between detrusor contractions and sphincter relaxation is more common in high, cervical cord transections and may increase maximal detrusor pressure in an attempt to overcome the functional bladder outlet obstruction. High, longstanding intravesical pressures, above 40 cm H_2O, are a foremost cause of upper urinary tract damage often resulting in renal failure (Ku, 2006). As most SCI patients do not fully recognize the degree of bladder filling, detrusor overactivity and high intravesical pressures are usually managed by rendering the detrusor arreflexic either by anti-cholinergic drugs, detrusor injections of botulinum toxin type A or dorsal sacral rhizotomies (Cruz and Dinis, 2007; Yoshimura, 2007). Patients will then periodically remove urine by self clean intermittent catheterization.

These therapies have important drawbacks. Orally active, anti-cholinergic drugs must be administered in high doses, above those recommended by the manufacturers, causing a considerable number of side-effects including dry mouth and constipation (De Groat and Yoshimura, 2001; Horstmann et al., 2006). In what concerns botulinum toxin type A injections, now that long-term efficacy of this treatment was established (Reitz et al., 2007; Del Popolo et al., 2008), the main problem resides in the necessity of performing repeated cystoscopies to inject the neurotoxin, increasing both the cost and the complexity of the treatment. Rhizotomies are a highly invasive procedure that definitely abolishes all remaining sensations from the perineal area.

Another strategy to deal with neurogenic bladder dysfunction is intravesical administration of capsaicin or resiniferatoxin (RTX). Its use is supported by the increased TRPV1 expression in urothelial cells and nerve fibres in the bladder of patients with spinal detrusor overactivity (Brady et al., 2004; Apostolidis et al., 2005). Nevertheless, in patients with high intravesical pressures intravesical vanilloids did not significantly reduce maximal detrusor pressure to safe levels, below 40 cm H_2O (Silva et al., 2000). In addition, although improving detrusor overactivity and urinary incontinence (Fowler et al., 1994; Cruz et al., 1997, Silva et al., 2000, 2005), intravesical vanilloids were less effective than botulinum toxin as they could not induce complete detrusor arreflexia (Giannantoni et al., 2004). Hence, the need for new more efficient therapies subsists.

Recently, it has been demonstrated that intrathecal and epidural administration of RTX produces strong enduring analgesia. In dogs with bone cancer pain, intrathecal injection of RTX improved the latency of paw withdrawal from heat (Brown et al., 2005). In rats epidural RTX also induced profound analgesia, which overcame that

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obtained by peripheral administration of the compound (Szabo et al., 1999). RTX effects were restricted to the injected segment and more selective than those observed after subcutaneous RTX injection (Karai et al., 2004). Side-effects in the 3 studies were minor. Thus, in the present study we aimed to investigate the effect of intrathecal administration of RTX in detrusor overactivity of rats with chronic spinal cord transection.

Materials and methods

Experimental animals

Adult female Wistar rats (220–250 g) from the IBMC colony (Porto, Portugal) were used. The ethical guidelines for investigation of experimental pain in animals (Zimmerman, 1983) and the European Communities Council Directive (86/609/EEC) were thoroughly followed in all procedures performed in this study. Thus, all efforts were made in order to minimise animal stress and suffering and to reduce the number of animals used.

Chemicals and reagents

All surgeries were performed under deep anaesthesia induced by intraperitoneal injection of a mixture of medetomidine 0.25 mg/kg and ketamine 60 mg/kg, in sterile saline. For cystometries and terminal handling, rats received as anaesthetic a subcutaneous bolus of urethane (1.2 g/kg). Resiniferatoxin (RTX) was purchased from Sigma (Portugal) and diluted in ethanol 10% in saline as vehicle. The antibody against TRPV1 receptor was custom made, raised in rabbits against the 15 C-terminal aminoacids of the rat TRPV1 sequence. Several tests to assure its specificity have been performed, including pre-absorption with excess cognate peptide, substitution for normal swine serum and staining of cord sections from TRPV1 knockout mice. In all occasions, no unspecific labelling was observed. The antibody against calcitonin gene related peptide (CGRP) was raised in rabbit (Chemicon, UK) whereas biotinylated isolectine B4 (IB4) was bought from Sigma (Portugal). The antibody against the active, that is phosphorylated, forms of ERK1 and 2 (phosphoERK) was from Neuromics (Germany) and it was produced in rabbit. The biotinylated swine anti-rabbit antibody and the avidin–biotin complex (ABC) conjugated with horseradish peroxidase were respectively bought from Dakopatts (Denmark) and Vector Laboratories (UK). The fluorocrome labelled antibodies used, donkey anti-rabbit Alexa Fluor™ 488 and Alexa Fluor™ 568 streptavidin, were purchased from Invitrogen (Portugal).

Experimental spinal cord transection (SCT) and catheter placement

A total of 20 animals were used for the SCT experiments. After induction of deep anaesthesia, a laminectomy was performed, after which the cord was sectioned at T7–T9 level and sterile gelfoam placed between the retracted ends of the cord. A silicone catheter (SF Medical; Hudson, USA) was placed into the lumbar subarachnoid space and the tip positioned at L6 level. The other tip of the catheter

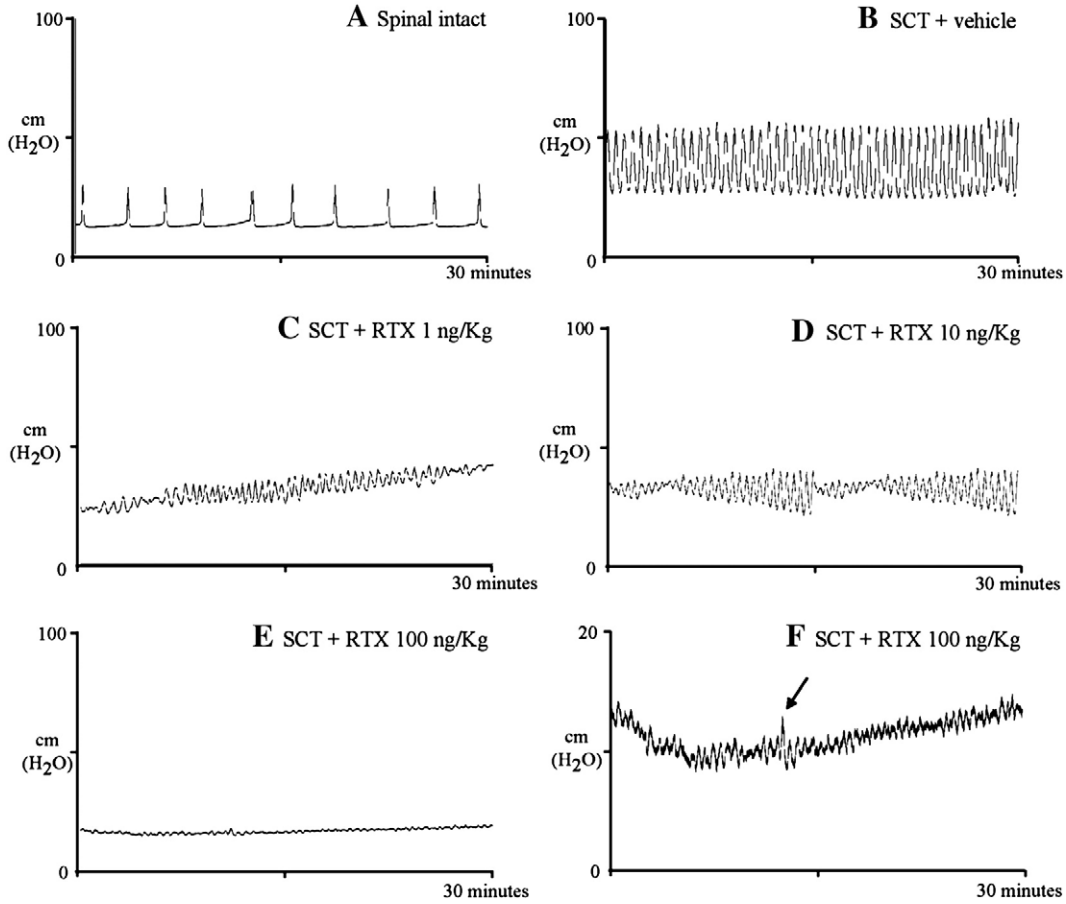

Fig. 1. Representative cystometrograms obtained from spinal intact and SCT animals. In (A), the frequency and amplitude of bladder contractions in spinal intact animals were not very high, the frequency of contractions being 0.47±0.09 per minute and the amplitude 7.24±1.62 cm H_2O. These values were considerably higher in non-injected and vehicle-injected (B) SCT rats. (C) Intrathecal injection of 1 ng/kg of RTX did not seemingly affect bladder overactivity. (D) Intrathecal delivery of 10 ng/kg of RTX in SCT rats resulted in less variable results. Although there was no reduction in the frequency of bladder contractions, their amplitude was reduced. (E) Injection of 100 ng/kg of RTX in SCT rats leads to drastic changes in bladder reflex activity of SCT animals. In fact, this amount of RTX totally abolished bladder contractions. (F) Magnification of the cystometrogram presented in (E). The arrow indicates one bladder contraction with amplitude of approximately 5 cm H_2O. All the other contractions did not reach this amplitude value.