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*Original article*

# The influence of resiniferatoxin on the chemical coding of neurons in dorsal root ganglia supplying the urinary bladder in the female pig

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## Abstract

Although resiniferatoxin (RTX) becomes more often used in experimental therapies of sensory system disorders, so far there is no data concerning the influence of RTX on the chemical coding of neurons in dorsal root ganglia (DRG) supplying the urinary bladder in the pig, an animal species considered as a reliable animal model for investigation dealing with human lower urinary tract disorders. Retrograde tracer Fast Blue (FB) was injected into the wall of the right half of the urinary bladder in six juvenile female pigs, and three weeks later, bladder instillation of RTX (500 nmol *per* animal) was carried out in all the animals. After a week, DRGs were harvested from all the pigs and the neurochemical characterization of $FB^+$ neurons was performed using routine single-immunofluorescence labeling technique on 10-µm-thick cryostat sections. RTX instillation resulted in a distinct decrease in the numbers of $FB^+$ cells containing calcitonin gene-related peptide (CGRP), nitric oxide synthase (NOS), somatostatin (SOM) and calbindin (CB) when compared with those found in the healthy animals (18% vs. 36%, 1% vs. 6%, 0.8% vs. 4% and 0.5% vs. 3%, respectively), and an increase in the number of pituitary adenylate cyclase-activating polypeptide (PACAP)- and galanin (GAL)-immunoreactive (IR) nerve cells (51% vs. 26% and 47% vs. 6.5%). The results obtained suggest that RTX could be taken into consideration when the neuroactive agents are planned to be used in experimental therapies of selected neurogenic bladder illnesses.

**Key words**: resiniferatoxin, urinary bladder, sensory innervation, dorsal root ganglia neurons, immunohistochemistry, neuropeptides, pig

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## Introduction

The storage and periodic elimination of urine requires a complex neural control system that coordinates the activities of a variety of effector organs including the smooth muscle of the urinary bladder (de Groat and Steers 1990). One of the crucial elements of reflex arc, participating in the regulation of the urinary bladder physiological functions, are DRGs sensory neurons. It has been shown in the past that in different species, including the rat, guinea pig, cat and pig (Habler et al. 1990, Callsen-Cencic and Mense 1997, Zhou and Ling 1997, Bossowska et al. 2009), the urinary bladder receives afferent innervation from sensory neurons located in thoraco-lumbar (rat and cat), lumbar (guinea pig and pig), lumbar-sacral (rat and guinea pig), sacral (cat) or sacro-coccygeal DRGs (pig) ganglia. Thus, urinary bladder-projecting sensory neurons occurring in two different locations, probably transmit stimuli that leads to a regulation of the urinary bladder activity. This assumption is indirectly supported by the differences in the chemical coding of DRGs neurons located at the lumbar and the sacro-coccygeal spinal cord levels: for example, much more lumbar afferent neurons associated with the porcine urinary bladder express CGRP, GAL, NOS and SOM, while most of those located in sacro-coccygeal DRGs are found to be PACAP-positive (Bossowska et al. 2009).

It is well-known that different bladder disorders in humans are associated with several distinct changes in urinary bladder functions and sensory sensations conveyed from the organ. The neuronal and mechanical events associated with bladder filling and micturition become hypersensitive and progressively painful, and neurogenic mechanism for the induction of an unstable and hyperreflexic detrusor muscle was postulated (McMahon and Abel 1987). It has also been suggested that detrusor hyperexcitability is mainly due to alterations in the afferent innervation of the bladder, mainly connected with changes in neurotransmitters expression in bladder sensory neurons. For example, it has been shown in rats that the induction of cystitis leads to a significant increase in the number of SP-, CGRP-, PACAP-, GAL- and NOS-immunoreactive (IR) bladder afferent nerve cells (Callsen-Cencic and Mense 1997, Vizzard 2000, 2001). Thus, taking into consideration that the hyperactivity or an abnormal activity of separate elements of the nervous system participating in the regulation of the lower urinary tract functions may be the main reason of numerous urinary bladder disorders, several neurotoxins have been introduced in experimental therapies of such illnesses. An example of such neurotoxin is RTX, isolated from the latex of several members of the genus *Euphorbia* (Szallasi and Blumberg 1989). Its mechanism of action consists on a blockade of the vanilloid receptor type 1 (VR1) in a subpopulation of primary afferent sensory neurons involved in nociception what, in turn, leads to abolishing the activity of afferent cells involved in abnormal neural circuits causing micturition disorders during various neurogenic diseases like overactive bladder or interstitial cystitis. It has been shown in a series of experiments that in adult rats a systemic administration of RTX up-regulates GAL and VIP (Farkas-Szallasi et al. 1995) and down-regulates SP and CGRP (Szolcsanyi et al. 1990, Szallasi et al. 1999) in primary afferent neurons, while intravesical treatment with RTX leads to a transient decrease in the number of SP- and CGRP-IR sensory fibers in the rat bladder wall (Avelino and Cruze 2000), but increases the number of GAL-IR nerve cells in L6 DRG of this species (Avelino et al. 2002). Thus, although there is clear-cut evidence that RTX is able to profoundly change the chemical phenotypes of urinary-bladder-projecting DRG neurons in rodents, there is, until now, no available data concerning the influence of RTX on the chemical coding of sensory DRG neurons supplying the urinary bladder in the pig, an animal species that can be used as a good animal model for investigations dealing with human lower urinary tract disorders. Therefore, the present study was aimed, by means of combined retrograde tracing and immunochemistry techniques, at determining the RTX-induced changes in the chemical coding of porcine urinary bladder-projecting DRG neurons.

## Materials and Methods

Data concerning the distribution pattern and chemical coding of the porcine DRG neurons projecting to the urinary bladder under physiological conditions were presented previously (Bossowska et al. 2009). It should be stressed that the number, sex, body weight and age of the animals used in the mentioned study as well as in the present experiment were fully matched. The present study was performed on six sexually immature Great Polish White female pigs (aged 8-12 weeks, 15-20 kg b.w.), kept under standard laboratory conditions with free access to food and water. Surgical procedures were applied in agreement with the guidelines of the Local Ethics Committee under deep thiopental anesthesia. All the animals were pretreated with atropine (Polfa, Poland; 0.04 mg/kg b.w., s.c.) and propionylpromasine (Stresnil, Janssen Pharmaceutica, Belgium; 0.5 mg/kg b.w., i.m.) thirty minutes before the main anesthetic, sodium thiopental (Sandoz, PL, 0.5 g per animal) was given intravenous-

ly in a slow, fractionated infusion. After a midline laparotomy, the urinary bladder was gently exposed and a total volume of 40 µl of 5% aqueous solution of the fluorescent retrograde tracer FB (Dr K. Illing KG & Co GmbH, Gross Umstadt, Germany) was injected into the wall of the right side of the bladder performing multiple injections. Three weeks later the bladder instillation of RTX (500 nmol per animal) was carried out in all the pigs. A week later all the animals were killed by an overdose of sodium pentobarbital and, after the cessation of breathing, perfused transcardially with freshly prepared 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Bilateral spinal ganglia of interest, together with the spinal cord were collected from all the pigs, postfixed in the same fixative for 10 minutes, washed several times in 0.1 M phosphate buffer and stored in 18% buffered sucrose at 4°C until sectioning. Ten-µm-thick serial cryostat sections, prepared from all DRGs studied were examined using an Olympus BX51 fluorescence microscope equipped with an appropriate filter set. Only $FB^+$ neurons with clearly visible nuclei were counted in every fourth section. The number of $FB^+$ cells found in all DRGs from the particular animal as well as the relative frequency of perikarya belonging to the particular neuronal classes were pooled and presented as mean ± SEM. The diameter of perikarya studied was measured by means of an image Analysis software (version 3.02, Soft Imaging System, GER) and data were used to divide urinary bladder-projecting neurons into the three size-classes: small (average diameter up to 30 µm), medium-sized (diameter 31-50 µm) and large afferent cells (diameter > 51 µm). The tissue sections were processed for immunohistochemistry, applying a routine single-labeling immunofluorescence technique, to investigate the presence of some biologically active substances including SP (rat monoclonal, Biogenesis, UK, 1:300), CGRP (rabbit polyclonal, Peninsula, USA; 1:9000), SOM (rat monoclonal, Biogenesis, UK; 1:60), GAL (rabbit polyclonal, Peninsula, USA; 1:1000), PACAP (rabbit polyclonal, Peninsula, USA; 1:15000), nNOS (mouse monoclonal, Sigma, USA; 1:400) and CB (rabbit polyclonal, Swant, Switzerland; 1:9000) in FB-labeled neurons. Briefly, after immersion in a blocking and permeabilizing solution containing 1% Triton X100, 0.1% bovine serum albumin, 0.05% thimerosal, 0.01% $NaN_3$ and 10% normal goat serum in 0.01M phosphate-buffered saline for 1 hour (h) at room temperature to reduce non-specific background staining, the sections were incubated overnight at room temperature with the particular primary antiserum in a humid chamber. Primary antisera were visualized by rat- and mouse-specific secondary antisera conjugated to FITC or rabbit-specific antibodies conjugated to biotin (all from Jackson Immunochemicals, USA). The latter antibodies were then visualized by a streptavidin-CY3 complex (Jackson Immunochemicals, USA). Control slides were processed as described, however, without incubation with the primary antibody. Retrograde labeled/single-immunostained perikarya were then evaluated under Olympus BX51 microscope equipped with epi-fluorescence and appropriate filters sets, counted in each fourth section (only neurons with clearly visible nucleus were included) and presented as mean ± SEM. Pictures were captured by a digital camera connected to a PC, analyzed with Analysis software (version 3.02, Soft Imaging System, GER) and printed on a wax printer (Phaser 8200, Xerox, USA).

## Results

There were no distinct changes in the number of SP-IR $FB^+$ neurons (49 ± 0.6%; Fig. 1a) after RTX treatment, when compared to that obtained under physiological conditions (45.2 ± 4.4%). In similarity to healthy animals (89.7 ± 1.3%), immunoreactivity to SP was found mainly in small-sized sensory cells (91.3 ± 2.9%). Furthermore, no differences in the number of bladder sensory nerve cells containing SP were observed between lumbar (43.7 ± 10.7%) and sacro-coccygeal (46.2 ± 6.0%; Fig. 1b) DRGs studied, when compared with data obtained from the animals investigated under physiological conditions (45.9 ± 4.6% and 44.2 ± 2.9%; Bossowska et al. 2009).

Although a distinct increase in the number of $FB^+$ neurons immunolabeled for PACAP (51.2 ± 8.5% vs. 26.1 ± 3.3%; Fig. 3a) was found after RTX treatment, it should however be stressed that in comparison with previous data (Bossowska et al. 2009) such increase was primarily observed in the lumbar population (64.5 ± 1.5% vs. 22.8 ± 4.6%) while in sacro-coccygeal DRGs, retrogradelly labeled sensory nerve cells responded to RTX treatment in a less pronounced manner (42.1 ± 11.7% vs. 31.2 ± 4.0%). Similarly to data obtained in the group of healthy animals (78.9 ± 2.1%), RTX-challenged PACAP-positive bladder sensory neurons belonged mainly to the class of small-sized perikarya (88.0 ± 3.6%; Fig. 3b-arrow).

The number of GAL-containing $FB^+$ sensory neurons (Fig. 4a,b) was distinctly increased (46.8 ± 18.4%) after bladder instillation with RTX, when compared to that obtained under physiological conditions (6.5 ± 2.5%). The majority of GAL-IR bladder-projecting cells (76.6 ± 1.9%) were small in a diameter, however, in contrast to the results obtained in the intact animals (Bossowska et al. 2009) a slight population of medium-sized GAL-IR neurons

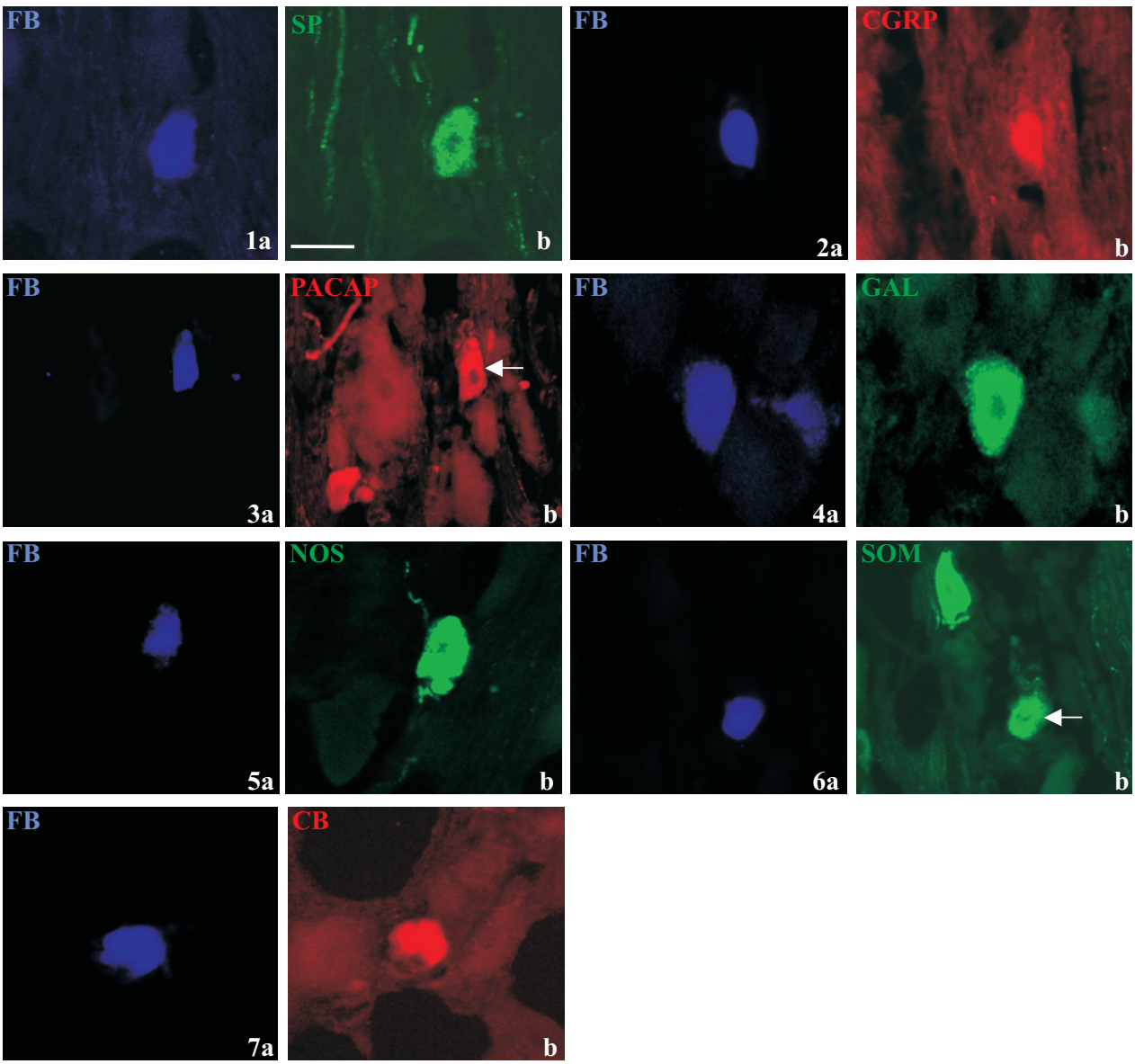


Fig. 1. S4 dorsal root ganglion (DRG), ipsilateral to the site of FB injection. Retrogradely traced bladder sensory neuron (**a**) containing SP-immunoreactivity (**b**). Scale bar = 50μm, apples to all figures; Fig. 2. L5 DRG, contralateral to the site of FB injection. FB-labeled nerve cell of a small diameter (**a**) exhibiting CGRP-immunoreactivity (**b**); Fig. 3. S3 DRG, contralateral to the site of FB injection. $FB^+$ afferent small-sized neuron (**a**) expressing PACAP-immunoreactivity (**b;** arrow); Fig. 4. L4 DRG, ipsilateral to the site of FB injection. Sensory neuron supplying the bladder wall (**a**) containing GAL-immunoreactivity (**b**); Fig. 5. S4 DRG, ipsilateral to the site of FB injections. A single medium-sized FB-traced sensory neuron (**a**) exhibiting NOS-IR (**b**); Fig. 6. Ipsilateral L5 DRG. The small FB-positive neuron (**a**) expressing SOM- immunoreactivity (**b;** arrow); Fig. 7. Ipsilateral S3 DRG. A single medium-sized FB-traced sensory neuron (**a**) exhibiting CB-IR (**b**).

was also observed after RTX treatment (23.4 ± 3.4%). It was previously found in healthy pigs (Bossowska et al. 2009) that the number of GAL-IR $FB^+$ cells was higher in the lumbar (9.3 ± 3.8%), than in the sacro-coccygeal subpopulation of bladder-projecting DRGs neurons (5.9 ± 2.4%); a similar relationship was observed after RTX administration (64.7 ± 10.2% vs. 35.2 ± 21.1%, lumbar vs. sacro-coccygeal neuronal subset, respectively). However, it should be stressed that a distinct increase in the numbers of GAL-IR cells was observed in both these neuronal subpopulations in the animals challenged by RTX instillation. In contrast to the increase in the number of neurons expressing PACAP or GAL immunoreactivities, RTX treatment resulted in a decrease in the number of $FB^+$ neurons containing CGRP, nNOS, SOM or CB (see below for details).

RTX-instillation of the bladder evoked an evident

decrease in the number of FB-labeled CGRP-IR neurons (18.4 ± 5.0% vs. 36.1 ± 4.7%). In contrast to results obtained in intact animals (Bossowska et al. 2009), immunoreactivity to CGRP was mainly observed in small-sized sensory neurons (90.8 ± 3.9% vs. 32.7 ± 2.1%; Fig. 2a), while this neuropeptide was not found in large $FB^+$ cells after RTX treatment. In similarity to the healthy animal group (Bossowska et al. 2009), more $FB^+$/CGRP+ were found in lumbar (43.7 ± 10.7% vs. 44.4 ± 4.3%; Fig. 2b) than in sacro-coccygeal DRGs studied (13,8 ± 5,1% vs. 23.5 ± 5.9%) but after RTX treatment there was observed a distinct decrease in the number of CGRP-IR cells in the sacro-coccygeal population of bladder sensory neurons.

A distinct decrease in the number of nNOS-IR $FB^+$ neurons was found after RTX treatment (1.1 ± 0.5% vs. 5.8 ± 2.5% observed under physiological conditions; Bossowska et al. 2009), particularly in the sacro-coccygeal subpopulation of bladder-projecting neurons (Fig. 5b). In accordance with the previous report (Bossowska et al. 2009), immunoreactivity to nNOS was primarily found in medium-sized retrogradelly labeled DRG neurons (78.3 ± 1.8% vs. 69.8 ± 2.6%; Fig. 5a). It should be stressed that after RTX treatment, nNOS-containing bladder-projecting cells were distinctly much more numerous in the lumbar (25.5 ± 7.5%) than in the sacro-coccygeal (0.2 ± 0.2%) subset of the bladder-projecting primary sensory nerve cells.

RTX treatment of the bladder led to a distinct decrease in the number of all the $FB^+$ SOM-positive neurons (0.8 ± 0.8% vs. 3.7 ± 2.8%, RTX-challenged vs. intact animals, respectively; Bossowska et al. 2009). However, this decline in the number was restricted exclusively to the lumbar population of bladder-projecting DRG neurons (Fig. 6b), while, in similarity to the intact animals (Bossowska et al. 2009), the lumbar subpopulation of SOM-IR bladder-projecting cells was still more numerous than that found in the sacro-coccygeal DRGs studied (4.5 ± 4.5% vs. 0.5 ± 0.9%, respectively). The majority of SOM-IR $FB^+$ cells (64.8 ± 2.3%) belonged to the class of small-sized perikarya (Fig. 6a), however, in contrast to the intact animals (Bossowska et al. 2009), a subpopulation of medium-sized $FB^+$ SOM-IR neurons (35.2 ± 1.4%) were also found after RTX treatment.

The number of $FB^+$ neurons containing CB was distinctly decreased (till 0.5 ± 0.6%;) after bladder instillation with RTX, when compared to the relative frequency of such coded neurons in the healthy animals (2.8 ± 1.4%; Bossowska et al. 2009) The majority of CB-IR bladder-projecting cells (83.9 ± 2.8%) were of medium size (Fig. 7a), however, in contrast to the data obtained under physiological conditions (Bossowska et al. 2009) several small-sized CB-IR neurons were also observed (16.1 ± 1.5%). While RTX instillation evoked a large decrease in the number of sacro-coccygeal bladder-projecting CB-IR DRG cells (0.5 ± 0.5% vs. 3.5 ± 1.2%; Fig. 7b), the number of CB-containing neurons within the lumbar subpopulation of sensory bladder-projecting nerve cells remained, in fact, unchanged after the exposition of the organ to the toxin (2.6 ± 2.2% vs. 3.1 ± 2.5%; RTX treatment vs. physiological conditions, respectively).

## Discussion

In the present study, an RTX-induced up-regulation in the expression of PACAP and GAL and a simultaneous down-regulation of CGRP-, nNOS-, SOM- and CB-immunoreactivity was found in the porcine urinary bladder DRG afferent neurons. Moreover, we provided, for the first time, some evidence that the expression of these substances in bladder afferent neurons can differently be up- or down-regulated in dependence on the spinal cord segmental level at which the parental DRGs were located. In general, it is now accepted that reflex contractions of the bladder are elicited by an activation of parasympathetic preganglionic neurons located in the sacral parasympathetic nucleus at the sacro-coccygeal spinal cord (segments S3 to Cq1 in the pig; see Bossowska et al. 2009), while an activation of sympathetic preganglionic neurons in the lumbar spinal cord (L3-L6 in the pig; see Bossowska et al. 2009) has inhibitory effects on bladder smooth muscle activity (Vaughan and Satchell 1995).

An intravesical administration of RTX was not able to evoke any distinct changes in the number of SP-IR bladder-projecting afferent neurons in the lumbar as well as sacro-coccygeal DRGs studied. This observation corresponds well with results obtained in the rat, where RTX treatment was also not able to evoke up- or down-regulation in the number of $SP^+$, bladder-projecting DRG neurons (Szallasi et al. 1999). It appears possible that SP may be involved in mediating urinary bladder hyperreflexia (Callsen-Cencic and Mense 1997) and neurogenic inflammation (Lundberg 1996). Furthermore, it has also been demonstrated that upon noxious stimulation in the periphery, SP is released from the central endings of DRG neurons (Daggan et al. 1995). Moreover, as central branches of SP-IR bladder-projecting DRG neurons were shown to project to the dorsal part of the sacral parasympathetic nucleus (Vizzard 2001), it may be suggested that this neuropeptide can be involved as an excitatory neurotransmitter in several types of bladder reflexes in the rat (Mersdorf et al. 1992). Thus, as may

be judged from the above-mentioned studies, it appears possible that also in the pig substance P may be involved in the regulation of urinary bladder functions at different levels of the neuraxis. However, the physiological relevance and the exact mechanism(s) and place(s) of action(s) of this neuropeptide in the domestic pig remains to be addressed in detail.

Although the exact physiological function(s) of PACAP in the lower urinary tract are still unclear yet, Ishizuka and co-workers (1995) reported that PACAP may be involved in the facilitation of spontaneous bladder contractions in rats, due to its excitatory action on the spinal micturition pathway. It has also been shown that the expression of PACAP in L1, L2, L6 and S1 DRG cells increased after chronic cyclophosphamide-induced cystitis (Vizzard 2000) in rats. These results suggest that PACAP may be a principal component of bladder hyperreflexia, leading to an increase in the excitability of sensory neurons participating in the bladder reflex arc. In addition, two supplementary functions have been postulated for PACAP in the micturition reflex pathways, including modulation of nociceptive transmission through interaction with NMDA receptors (Oshawa et al. 2002) and/or modulation of inflammatory responses (Kim et al. 2000) by downregulation of inflammatory cytokines including tumor necrosis factor-alpha. It has also been shown in pigs that PACAP produces relaxation in the urinary tract (Hernandez et al. 2006). In the present study, RTX led to a distinct increase in the number of PACAP-IR bladder-projecting sensory neurons in the lumbar as well as sacro-coccygeal DRGs studied. As may be judged from studies reporting that RTX action on overactive bladder or interstitial cystitis-challenged organ is probably based on the inhibition of pain sensation and frequency of voiding (Cruz and Dinis 2007, Mourtzoukou et al. 2008) it appears possible that in pathological cases PACAP participates in the modulation of nociceptive transmission and urinary bladder relaxation.

In the present study we provided some evidence that the number of GAL-expressing bladder-projecting sensory neurons distinctly increased in lumbar as well as in sacro-coccygeal DRGs after bladder instillation of RTX. A very similar increase in the number of GAL-IR bladder afferent neurons was observed in the rat after intravesical RTX treatment (Avelino et al. 1999). In all animal models of neuropathic pain evoked by peripheral nerve injury, GAL not only was upregulated in DRG neurons, but also an increase in its release has been reported in superficial layers of the spinal dorsal horn (Colvin et al. 1997). This leads to an activation of the inhibitory GAL1 receptors on putatively glutamate-containing excitatory dorsal horn neurons and thus to attenuation of pain transmission (Liu and Hokfelt 2002). Furthermore, as GAL antagonized facilitatory effect of SP on the nociceptive flexor reflex in the rat (Xu et al. 1989) and has been shown to play a tonic inhibitory role in the mediation of spinal cord excitability, it may be speculated that the increased expression of GAL after bladder instillation with RTX contributes to the state of desensitization by antagonizing the effect of SP released from bladder fibers in the spinal cord (Lecci et al. 1993).

A distinct decrease in the number of FB-labeled CGRP-IR neurons, especially within the sacro-coccygeal population of these perikarya, has been observed after bladder instillation with RTX. Previous studies have suggested that CGRP, which *per se* has no excitatory effect on the vesico-vesical reflex pathway (Maggi 1990), is able to facilitate the SP-evoked chemonociceptive reflex. CGRP acts synergistically with SP in the spinal cord (Biella et al. 1991) and such synergism may result from CGRP-mediated inhibition of an endopeptidase that degrades SP (Le Greves et al. 1985), thus elevating the local concentration of SP at the site of release. It has been shown that cystitis leads to a significant rise in the number of CGRP-IR bladder-projecting cells in rats (Callsen-Cencic and Mense 1997, Vizzard 2001). This may be suggestive for an involvement of CGRP in the sensitization of afferent neuronal pathways in the lower urinary tract and for a role of CGRP in mediation of bladder overactivity (Vizzard 2001). Thus, the decrease in the number of CGRP-IR bladder primary afferent neurons after RTX instillation, what probably leads to a diminishing of its release within the sacral spinal cord, might reduce the excitatory effect of SP on the preganglionic neurons forming parasympathetic nucleus, what in turn, may lead to an inhibition of bladder contractions and pain transmission under pathological conditions.

Bladder instillation with RTX has induced a distinct decrease in the number of nNOS-containing, FB-labeled neurons, especially in the sacro-coccygeal population of the nerve cells. Interestingly, an opposite phenomenon was observed in rats, in which the systemic RTX treatment triggered off an increase in the number of nNOS-IR DRG neurons (Farkas-Szallasi et al. 1995). This discrepancy is probably caused by different ways of RTX application. It has been shown that NO may act as “retrograde transmitter” in the sensory pathways and plays a pivotal role in nociceptive processing in multisynaptic local circuits of the spinal cord (Meller and Gebhart 1993). It has also been suggested to play a role in the facilitation of the micturition reflex evoked by noxious chemical irritation of the bladder as well as to be involved at the spinal level in the facilitation of the micturition reflex by nociceptive bladder afferents (Kakizaki and de

Groat 1996). As RTX produced a significant decrease in the number of NOS-IR bladder sensory neurons in the pig, it may be suggested that this neurotoxin reduces the excitatory effect of NOS at the spinal level, acting on the population of preganglionic neurons located in the parasympathetic sacral nucleus, what in turn, may cause an inhibition of bladder contractions and pain transmission.

After RTX treatment, a distinct decrease in the number of SOM-IR bladder neurons was observed in the porcine lumbar DRGs studied. It was shown in the previous study that RTX interfered with SOM release from capsaicin-sensitive sensory nerve terminals in rats (Happelmann and Pawlak 1997). This observation suggests that RTX can also increase SOM release from (putatively) capsaicin-sensitive sensory nerve terminals in pigs, what in turn leads to a decrease in the number of bladder sensory neurons containing SOM in an amount being detectable by immunofluorescence technique used. It is well known that SOM exerts a systemic antinociceptive effect (Helyes et al. 2000) and inhibitory action on acute neurogenic and non-neurogenic inflammatory reactions (Than et al. 2000).

CB is thought to act as a $Ca^{2+}$ buffer, thus controlling $Ca^{2+}$ levels within the cytoplasm of a nerve cell. CB has been reported to act not only as a passive buffer but also to have active roles in neuronal activity (Baimbridge et al. 1992). CB was previously found in medium- or small-sized DRG neurons, mostly in a subpopulation of muscle and visceral nociceptors (Honda 1995). Numerous CB-containing small- or medium-sized DRG neurons also contained SP and co-expressed vanilloid receptor type 1 (Li et al. 2005). This strongly suggests that CB is involved in some aspects of pain transmission. In pigs, the number of bladder sensory neurons containing CB distinctly decreased in lumbar as well as in sacro-coccygeal DRGs after bladder instillation of RTX. It probably may lead to changes in CB control action on the $Ca^{2+}$ homeostasis in affected neurons, what, in turn, results in a decrease in SP release rate from central afferent branches in the dorsal horn, suppressing pain transmission.

The present study has revealed that RTX profoundly influences the chemical coding of DRG nerve cells supplying the porcine urinary bladder. This strongly suggests that RTX can be used in case of hyperactivity of the afferent limb of reflex arcs responsible for transmission of sensory and pain information from the urinary bladder, and that the influence of RTX on the sensory bladder innervation has to be taken into account, when this neuroactive agent is used in the experimental therapy of selected neurogenic bladder illnesses.

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