WORKSHOP M9 — THE VANILLOID CLUB

Chair: Francisco Cruz, Clare Fowler

ROLE OF TRPV1 (VR1) IN BLADDER NEURAL-EPITHELIAL SIGNALING

Lori A. Birder, University of Pittsburgh School of Medicine, Departments of Medicine Renal-Electrolyte Division and Pharmacology, Pittsburgh, PA, USA

Recent studies from our laboratory have revealed that the urinary bladder (UB) epithelium (urothelium) is involved in sensory mechanisms and can release chemical mediators (nitric oxide, NO; ATP). Localization of afferent nerves next to the urothelial cells suggests that these cells may be targets for transmitter release from bladder nerves or that chemicals released by urothelial cells may alter afferent excitability. These findings suggest a bi-directional chemical communication between the urothelium and adjacent nerves and that alterations in afferents or epithelial cells in pelvic viscera may contribute to the sensory abnormalities in pelvic disorders.

Painful sensations induced by capsaicin, the pungent substance in hot peppers, are due to stimulation of vanilloid receptor 1 (TRPV1), a cation channel expressed by primary afferent neurons of the “pain” pathway. TRPV1 also participates in the detection of at least two additional noxious stimuli, acid (pH <6) and heat (>43°C). The urinary bladder is richly innervated by capsaicin-sensitive afferent fibers that detect bladder distension or the presence of irritant chemicals. Activation of these afferent fibers can in turn trigger reflex bladder activity. Still, the contribution of TRPV1 to bladder physiology remains unclear. Recently we have explored the significance of this expression by analyzing bladder function in mice lacking TRPV1.

We have evidence that TRPV1 is expressed not only by afferent nerves that form close contacts with bladder epithelial (urothelial) cells, but also by the urothelial cells themselves. The urothelium is a multilayered structure consisting of basal cells, intermediate cells and large superficial cells. Immunostaining revealed TRPV1-IR in both basal and superficial epithelial cells. These studies also revealed that TRPV1-IR nerve fibers were localized throughout the urinary bladder musculature and in a plexus beneath and extending into the epithelium.

We further showed that exogenously applied vanilloids increase intracellular Ca^{2+} and evoke nitric oxide (NO) and ATP release in urothelial cells and that these responses require TRPV1. These and other data suggest that urothelial cells work in concert with underlying afferent nerves to detect the presence of irritant stimuli. In addition, the functional response of endogenous urothelial cell vanilloid receptors, like that of recombinant TRPV1, can be modulated by protons. Demonstration of a TRPV1 receptor in urothelial cells sensitive to capsaicin and protons suggest that TRPV1 in non-neuronal cells may have a role in sensory mechanisms in the urinary bladder.

To explore the physiological significance of TRPV1 in the bladder, we examined urinary tract anatomy and function in mice in which the TRPV1 gene had been disrupted. Bladder contraction frequency was unaffected by capsaicin in TRPV1 null mice. However, compared with wild-type littermates, TRPV1 null mice exhibited an increased frequency of “non-voiding” bladder contractions (intravesical pressure waves whose amplitudes were greater than 15 cm H_2O and did not result in obvious voiding), with no change in the frequency of “voiding” contractions. Under anesthesia, TRPV1 null mice also exhibited an abnormally increased bladder capacity and reduced induction of spinal cord c-fos protein upon bladder filling. Defective voiding responses were not confined to mechanical stimuli. We evaluated bladder responses to two chemical stimuli (capsaicin and protons) known to activate TRPV1. Intravesically infused capsaicin, (50-100 µM), significantly decreased the ICI (52 $\pm$ 8%) in wild type mice, but did not significantly change the ICI in mice lacking TRPV1, demonstrating that TRPV1 indeed mediates this effect. By comparison, subsequent infusion of acetic acid decreased ICI to a similar extent (mean 70%) in both wild type and null mice.

A number of studies have implicated extracellular ATP, most likely of urothelial origin, in distension-evoked activation of bladder afferents. The cellular mechanism(s) by which stretch evokes the release of ATP from urothelial, endothelial, or other epithelial cell types is unclear. One possibility is that cellular distension causes intracellular vesicles rich in ATP to fuse with the plasma membrane. Consistent with this possibility, mechanical distension of the excised rabbit urinary bladder has been shown to trigger an increase in the membrane capacitance of the apical urothelial surface. In vitro, stretch-evoked ATP release and membrane capacitance changes are reduced in bladders excised from TRPV1 null mice, as is hypotonic stretch-evoked ATP release from cultured TRPV1 null urothelial cells. Thus, TRPV1 appears to participate in normal bladder function, and is essential for normal mechanically evoked purinergic signalling by the urothelium.

Our data, together with previously reported findings, support the idea that urothelial cells exhibit specialized sensory and signalling properties that allow them to respond to their chemical and physical environments and engage in reciprocal communication with neighbouring nerves in the bladder wall. These properties include: (1) the expression of nicotinic, muscarinic, tachykinin and adrenergic receptors as well as vanilloid receptors; (2) responsiveness or sensitivity to transmitters (ATP, Substance P) released from afferent

nerves; (3) close physical association with afferent nerves; and (4) the ability to release chemical mediators such as ATP and NO that can regulate the activity of underlying nerves and thereby trigger local vascular changes and/or reflex bladder contractions.

Together, our findings demonstrate that the functional significance of TRPV1 in the bladder extends beyond pain sensation to include the regulation of voiding. Given that topical treatment with capsaicin improves urinary continence and relieves pain in humans with bladder hypersensitivity, these observations further suggest that TRPV1 represents a promising target for the study of pathological bladder function.

VANILLOID RECEPTORS: MORE THAN JUST NERVE FIBERS

Dirk De Ridder, Frank Van der Aa, Dieter Ost, Tania Roskams

Introduction: In the human bladder, the vanilloid receptor is thought to be present on small to medium-sized unmyelinated afferent C-type neurons. Animal experiments in a cat model have demonstrated that when spinal cord injury (lesion, multiple sclerosis etc…) occurs, the afferent limb of the sacral micturition reflex induces rhythmic hyperreflexic bladder contractions[1]. Vanilloids are thought to selectively block the C-fibres by desensitisation[2]. The decrease in density of small unmyelinated nerve fibres in the suburothelium in patients responding to treatment with vanilloids supports this theory[3]. This mechanism however fails to explain the 20-30% failure after intravesical treatment in patients with spinal cord disease. Probably the working mechanism is more complex.

So far the topography of the vanilloid receptor in the human bladder has mainly been studied using indirect methods such as autoradiographic visualisation[4] and immunohistochemical stainings for calcitonin gene related peptide and substance P. More recently the vanilloid receptor has been cloned[5] and antibodies have been generated.

We studied the presence and distribution of the vanilloid receptor in the human urinary bladder to confirm or reject previous findings by other groups that used indirect methods or VR-1 antibodies made by immunizing experimental animals.

Materials and methods: Eleven normal urinary bladder tissues samples were obtained from cystectomy specimens and fresh frozen processed. The specimens were studied by immunohistochemistry and confocal lasermicroscopy using three different VR-1 antibodies. Immunohistochemical colocalisation studies for neurofilament, nNOS and nerve growth factor were performed

Results: All biopsies were normal on routine HE staining. The antibodies raised against the C-terminus of the receptor (both from Chemicon®) showed a consistent staining pattern. Non-myelinated nerve fibres as well as myelinated nerve fibres were immunoreactive throughout the biopsies. There was unexpected staining of smooth muscle cells. The two antibodies directed against the C-terminus gave the same staining pattern, both with peroxidase and alkaline phosphatase labelling. Controls, consisting of omission of the primary antibody were negative. The suburothelium was dense immunoreactive, due to the presence of a dense immunoreactive network of unmyelinated nerve fibres. A high degree of immunoreactivity on interstitial cells was however also noted. No immunoreactivity was noted on the endothelium of the capillary arteries or on the urothelium. On three-dimensional confocal lasermicroscopy these data were confirmed, including the smooth muscle staining. When matching the vanilloid receptor antibody staining to the double staining with neurofilament, no staining of the nerves in close relation with the muscle cells was noted, but the VR- immunoreactivity is present on the smooth muscle cells themselves.

The antibody raised against the N-terminus (rabbit polyclonal aVRL11-P from Biotrend®) was identically processed. There was an abundant immunoreactivity on non-myelinated nerve fibres in the suburothelium and non-myelinated as well as myelinated nerve fibres throughout all muscular tissue. Interstitial cells were immunoreactive and present in a high proportion in the suburothelium and in fibromuscular axes in the muscle layer. There was an unexpected immunoreactivity on the endothelium of the capillary arteries. No reactivity was found on veins or lymph-vessels. There was no immunoreactivity at all at the smooth muscle cells on light microscopy. To determine whether this effect was due to sterical interference of the long molecular structure of Envision with surrounding structural proteins, classic immunohistochemical stainings using a three step unlabeled peroxidase-anti-peroxidase (PAP) method, as described previously[6] were performed. These stainings also showed no smooth muscle cell immunoreactivity.

Using the 3D confocal laser microscope, immunofluorescence was found on the smooth muscle cells both with the antibodies raised against the C-terminus and the N-terminus. When matching the confocal lasermicroscopic VR1 staining with NF, a perfect match was seen between all nerve fibres, but there was no match between NF and the smooth muscle cells. The same results were obtained using the antibody raised against the N-terminus.

Colocalisation studies for nNOS and NGF were performed using both antibodies. NGF did not have a specific pattern. nNOS colocalised perfect with VR-1 immunoreactive interstitial cells in the suburothelium and in fibromuscular axes in the muscular layer. Classic double staining slides with NF revealed no match with the immunoreactivity on the interstitial cells, but a perfect match between NF and all nerve fibres.

Conclusion: Our data partially confirm earlier findings. The vanilloid receptor is present on small unmyelinated (C-type) afferent and myelinated (Aδ-type) nerve fibers. The vanilloid receptor is also present on interstitial cells located in the suburothelium and the fibromuscular axes of the muscle layer. There is VR-immunoreactivity on the smooth muscle cells, but its physiological meaning is unclear.

The staining pattern obtained with the three VR-1 antibodies is not consistent, and depending on the terminus of the receptor against which it was raised, VR- immunoreactivity is present on the endothelium of capillaries (Ab raised against the N-terminus) or is not visible on light microscopy in the smooth muscle cells (Ab raised against N-terminus), possibly due to an isoform of VR-1.

These data also suggest that the current hypothesis about the mechanism of action of vanilloids through desensitization of the afferent c-fibres of the sacral reflex arc has to be revised. Local direct or indirect effects could be responsible for the clinical response of vanilloid treatment. A local interaction between urothelium, smooth muscle cells, myelinated and unmyelinated nerves and interstitial cells deserves further attention.

References

1. de Groat,W.C., Kawatani,M., Hisamitsu,T. et al. Mechanisms underlying the recovery of urinary bladder function following spinal cord injury. J.Auton.Nerv.Syst. Vol(30 Suppl), pS71, 1990.
2. Lynn,B. Capsaicin: actions on C fibre afferents that may be involved in itch. Skin Pharmacol. Vol(5), p9, 1992.
3. Dasgupta,P., Chandiramani,V.A., Beckett,A. et al. The effect of intravesical capsaicin on the suburothelial innervation in patients with detrusor hyper-reflexia. BJU.Int. Vol(85), p238, 2000.
4. Szallasi,A. Autoradiographic visualization and pharmacological characterization of vanilloid (capsaicin) receptors in several species, including man. Acta Physiol Scand.Suppl Vol(629), p1, 1995.
5. Tominaga,M., Caterina,M.J., Malmberg,A.B. et al. The cloned capsaicin receptor integrates multiple pain-producing stimuli [see comments]. Neuron Vol(21), p531, 1998.
6. Roskams,T., Willems,M., Campos,R.V. et al. Parathyroid hormone-related peptide expression in primary and metastatic liver tumours. Histopathology Vol(23), p519, 1993.

BLADDER STIMULATION OF VANILLOID RECEPTOR 1 AND REGIONAL CEREBRAL BLOOD FLOW CHANGES IN HUMANS: A SPET STUDY

Massimo Lazzeri, Damiano Turini, Department of Urology, University of Ferrara, Ferrara, Italy

Background. Although the brain plays an important role in the regulation of micturition in humans, little is known about the relationship between peripheral sensory innervation and cerebral control of voiding. During the last ten years many studies have been carried out on the spinal and local control of micturition showing a crucial role of primary sensory innervation. At the same time much clinical data has been collected on the effects of selective activation of Vanilloid Receptor 1 (VR1), which is expressed on peripheral endings of primary sensory nerves, by capsaicin and its ultrapotent analogue resiniferatoxin, in normal and pathological voiding reflex. However, little information is available concerning brain functioning during the peripheral selective activation of VR1.

Aim of the study. The objective of this study is to perform a qualitative and quantitative analysis, by SPET (Single Photon Emission Tomography), of regional cerebral blood flow (rCBF) changes during normal and capsaicin induced voiding reflex in normal and patholol gical conditions in humans.

Material and Methods. The study was performed on 7 normal subjects, 5 patients suffering from hypersensitive disorder of lower urinary tract (frequency/urgency syndrome plus/minus pelvic/perineal pain) and 3 patients with a sacral neuromodulator for idiopathic urinary retention. For each subject we performed three measurements: i) Basal recording; ii) basal micturition recording and iii) capsaicin induced micturition recording. SPET (Single photon emission tomography) assessment was performed 20 minutes after the intravenous (i.v.) bolus administration of 740 MBq of 99mTc-hexamethylpropylene amine oxime (HMPAO) in an antecubital prepared vein. The injection was performed by a self-injection device. The General Electric Millennium VG, a dual head variable angle large field of view gamma camera, was used for recording. It was fitted with parallel-hole low energy, high resolution collimators. The acquisition of one projection was obtained

for each 3° in a 360° orbit for a total of 120 projections. The subject's head was securely positioned in an adjustable head-holder device. A 128x128 matrix was used. The reconstruction was performed with an interactive reconstruction method, obtaining transaxial sections orientated in a fronto-occipital plane with slices/depth of 10 mm. Images were obtained in the axial, coronal and sagital views; color transformation was also made. A quantitative analysis was performed by the extraction of activity per region of interest (ROI) corrected for the injected activity. The ratio expressed the following formula in order to obtain an absolute number:

$$\frac{\text{average Region of Interest (ROI) counts/sec. x 100000}}{\text{brain counts/sec in anterior view}}$$

Count express radioactivity decay over the time (counts/sec.).

Results. Normal subjects. The qualitative analysis showed an increase of rCBF in four areas of the brain during normal micturition: the precentral supero-lateral gyrus, the precentral supero-medial gyrus, the right (left in the left-handed patient) anterior gyrus and the hypothalamus. Al these observations were confirmed by quantitative analysis. The same observations were recorded with capsaicin, with the exception of hypothalamus where no increase of rCBF was increased. The rCBF was statistically higher during normal micturition than during capsaicin induced voiding reflex in the pre-central supero-lateral gyrus ($p = 0.024$), in the right (left)-anterior gyrus ($p = 0,039$) and in the hypothalamus ($p = 2,93 \times 10^{-5}$), but not in the pre-central supero-medial gyrus ($p = 0,060449$). Patients with hypersensitive disorder. The qualitative analysis showed an increase of rCBF in the same four areas of the brain during the spontaneous micturition: the precentral supero-lateral gyrus, the pre-central supero-medial gyrus, the right anterior gyrus and the hypothalamus. The quantitative analysis showed a significant lower increase of rCBF in normal as well as capsaicin induced micturition for each cortical areas but not at the level of hypothalamus when the data were compared with normal patients. Patients with sacral neuromodulator. The qualitative analysis showed an increase of rCBF in the same previously four areas of the brain during the neuromodulator-ON micturition: the pre-central supero-lateral gyrus, the pre-central supero-medial gyrus, the right anterior gyrus and the hypothalamus. The quantitative analysis showed a significant higher increase of rCBF when patients voided with neuromodular-ON for each cerebral areas, when the data were compared with those from normal patients. Similar findings were recorded with capsaicin and neuromodulator-OFF. However, capsaicin produced a lower activation of cerebral areas in this group of patients.
Conclusion. Our findings show that the selective peripheral activation of VR1 produces modifications of rCBF in different areas in normal conditions as well as in pathological conditions. Our data support the idea of a highly complex voiding reflex and suggest that new investigations remain mandatory, in order to better understand the relationship between peripheral sensory innervation and rCBF in normal conditions and in lower urinary tract dysfunction.

RTX TRIAL EXPERIENCE

Ciaran M. Brady, Matthew Harper, Clare J. Fowler, Dept. of Uro-Neurology, National Hospital for Neurology & Neurosurgery, Queen Square, London, UK

Introduction

Studies in the rat have shown that following spinal cord injury, capsaicin-sensitive unmyelinated afferent C-fibres originating in the suburothelium exhibit somal hypertrophy and increased excitability, thought to be the underlying cause of neurogenic detrusor overactivity (NDO). Topical application of capsaicin or its ultrapotent analogue resiniferatoxin (RTX) to the serosal surface of the urinary bladder of adult rats results in early acute excitatory effects ("sensitisation") with facilitation of reflex bladder activity and behavioural changes indicative of bladder pain. This is followed by desensitisation, which may be defined as the long lasting, reversible suppression of sensory neuron activity. It is thought that the eventual recurrence of symptoms is related to C-fibre regeneration. In previous studies by Cruz, intravesical RTX in concentrations as low as 100 nMol induced full desensitisation whereas capsaicin required 1 mMol solutions to induce the same effect. In addition, 100 nMol RTX solutions were much less irritative to bladder afferents.

Clinical Trials of RTX

Between 1999 and 2000, we participated in one of a series of clinical trials of RTX (RTX107; Sponsor, Afferon Corporation). Patients with refractory NDO were randomised to receive a single 30 minute intravesical instillation of placebo, 0.1 uMol, 0.5uMol or 1uMol RTX, in a double blind fashion. In the absence of a clinical response to treatment, patients were retreated with the next highest dose, until a response was obtained or a

maximum of 2 treatments of 1uMol RTX had been given. During our study it became apparent that the response to treatment was unpredictable, despite the "dose escalation" design of the study.

RTX "adsorption"

Trials were suspended whilst further "pre-clinical" studies revealed that RTX adsorbs (sticks) to certain types of plastic. Although not every type of plastic has been investigated, the affinity of RTX for PVC is the same as for polypropylene. These materials were present in the fluid bags, giving sets, syringes and catheters used in the clinical trial. Further studies have revealed that adsorption of RTX to plastic is "incubation time" dependant and that the binding curves are fairly linear, with 50% binding of the drug taking 2 hours. Therefore, the actual dose of RTX received by each patient was determined by the length of time it was stored in the PVC containers prior to administration. Solutions were freshly prepared in the pharmacy, refrigerated and stored for up to 24 hours prior to dispensing. Stability studies revealed that rates of adsorption are essentially the same for solutions at room temperature or 4˚C.

Aim

We have previously shown that the mean suburothelial nerve density decreases in patients who respond to capsaicin. The aims of our study (M. Harper et. al., ICS 2002) included measurement of suburothelial nerve density in bladder biopsies from patients with NDO before and after intravesical RTX, taking the above issues into account.

Methods

Flexible cystoscopic biopsies were taken before and after intravesical RTX in 18 patients who received RTX for refractory NDO (13 had multiple sclerosis, 3 had spinal cord vascular accidents, 1 had tropical spastic paraparesis and 1 had spinal cord injury). Biopsies were taken 4 weeks following treatment or at a time where the clinical response was continuing. Clinical response was determined by analysis of voiding diary data. Multiple 10μM frozen sections were stained with anti bodies to the pan-neuronal marker PGP 9.5 for nerve density analysis. (Please see Harper et. al. ICS 2002, for further details)

Results

The mean (and median) total number of treatments of varying (escalating) doses of RTX received by clinical responders prior to biopsy was 3 (3) compared to 3.3 (4) in the non-responder group. In each case, 1uMol RTX was prepared in the pharmacy, with varying incubation times prior to RTX administration. Based upon these incubation times, the average (and median) dose of RTX was 0.41 (0.40) uMol in those who responded versus 0.37 (0.30) μMol in the non- responders. In those who responded clinically to RTX therapy (n= 5) the density of PGP 9.5 staining nerves in the suburothelium decreased from an average of 229.1 (SD 143.86) to 22.9 (SD 21.27) per mm^3 following various doses of RTX (p=0.033). In non-responders (n= 8), the nerve densities did not change significantly after treatment with RTX.

Conclusions

We have shown that intravesical RTX decreased the number of immunoreactive suburothelial nerves in patients who responded to treatment. It is unlikely that treatment response is related exclusively to the slightly lower doses of drug given to the non-responder group. Because disease type did not correlate with clinical outcome, it is necessary to identify other markers that might predict response to treatment. We believe that these results support further clinical trials of RTX. Future studies should aim to minimise the time RTX is exposed to plastic by mixing the drug in glass just prior delivery to the bladder. As the only contact with plastic is during the 5-15 minutes it takes to deliver the drug, the adsorptive properties of RTX should be negligible. Further immunohistochemical studies (e.g. Vanilloid receptor staining pre and post RTX) might help to clarify the mechanism of action of intravesical vanilloid therapy.

INTRAVESICAL RTX IN NEUROGENIC AND NON-NEUROGENIC BLADDER OVERACTIVITY

Francisco Cruz, Carlos Silva, Hospital S. João and Faculty of Medicine of Porto, Porto, Portugal

Resiniferatoxin (RTX), a VR1 agonist extracted from the dry latex of a cactus like plant, was introduced as a desensitizing agent of human bladder C-fibers due to its strong desensitizing power and weak excitation effect (Avelino et al.,'99, Cruz et al.,'97). The objective was to avoid bladder pain imputed to the strong C-fiber excitation that preceded capsaicin-induced desensitization and which mitigated the success of this agent in bladder overactivity of spinal origin (Fowler et al. 92, 94, Cruz et al.,'97, De Ridder et al.,'97). The low pungency of intravesical RTX allowed us to extend desensitizing therapy to idiopathic bladder overactivity, a mic-

turition disturbance where C-fiber input might also be involved in the genesis of involuntary detrusor activity.
RTX was obtained from Afferon (USA) or Sigma as a dry powder. A 10 μM stock solution in pure ethanol was prepared and kept in the dark at 4ºC in a glass container (1 mg RTX dissolved in 159 ml. of ethanol). Instillations were carried out with 100 ml. (or half of the bladder capacity) of 50 nM or 100 nM RTX solutions. 10% ethanol in saline was used as vehicle (preparation of 50 nM solution: 90 ml saline + 0.5 ml. stock solution + 9.5 ml. ethanol; preparation of 100 nM solution: 90 ml saline + 1 ml. stock solution + 9 ml. ethanol). Solutions were prepared before each instillation to reduce the contact of RTX with plastic containers as plastic polymers were shown to bind the molecule. Although the ideal vehicle for intravesical RTX application is yet to be defined, low concentrated ethanol solutions improve RTX solubility and might contribute to vanilloid receptor activation (Travisani et al.,'02). Instillations were always carried out without any form of bladder anaesthesia. A 3-ways urethral catheter was inserted in the bladder, the urine was emptied and RTX solution maintained in the bladder for 30 minutes. In the end the bladder was emptied and rinsed with saline, the catheter was removed and patients discharged home. During the procedure analgesics were never required by our patients, including those with idiopathic bladder overactivity which had normal bladder sensation. Episodes of autonomic dysrreflexia were not triggered in susceptible patients and no transient worsening of urinary symptoms occurred. Phasic detrusor contractions were frequent during RTX instillation although becoming more space to the end of the treatment. Leakage of RTX solution into the urethra was, however, prevented by maintaining the urethral catheter gently pulled against the bladder neck. In addition, the plastic container used for RTX transport was left attached to the urethral catheter to accommodate the bladder content during detrusor contractions.
The first non-controlled trial (Silva et al.,'00) was conducted in 14 spinal patients and improved or abolished incontinence in 75% of them. The effect was long-lasting, up to 12 months, in half of the patients. At 90 days urinary frequency decreased from 14.2 ± 6.4 to 10.3 ± 3.2 (p=0.01) whereas first detrusor contraction increased from 134.2 ± 83.1 ml to 186,4 ± 112.6 ml (p=0.05) and maximal cystometric capacity increased from 182.3 ± 119.8 ml to 330.0 ± 201.6 ml (p=0.01). Maximal detrusor pressure was not modified by the treatment.
In patients with idiopathic bladder overactivity RTX increased the volume to first detrusor contraction from 170 ± 109 ml to 440±130 ml (p=0.0001) at 30 days and to 391±165 ml (p=0.008) at 90 days. Maximal cystometric capacity also rose from 291 ± 160 ml to 472±139 ml (p=0.01) at 30 days and to 413±153 ml (p=0.1) at 90 days). Daily number of episodes of urinary incontinence decreased from 4.3 ± 2.7 to 0.9 ± 2.7 (p=0.001) at 30 days and to 0.7±0.9 (p=0.009) at 90 days. Frequency decreased from 12 ± 3.2 to 9.7±3.2 (p=0.003) and 9.9±3.5 (p=0.001) times per day at the same time points (Silva et al.,'02).
One caveat of intravesical RTX is the lack of placebo controlled studies. To fill this gap we initiated a clinical trial in patients with spinal bladder overactivity in which RTX is compared against the vehicle solution (10% ethanol in saline). Preliminary results show that discomfort using a 10 points visual analogue scale was 2.0± 2 after RTX and 0.7±3 after ethanol (ns). In the RTX group first detrusor contraction and mean maximal cystometric capacity rose from 143±105 ml to 187±109 ml (p=0.03) and from 186±109 to 332±142 ml (p=0,0001), respectively. In the ethanol group first detrusor contraction and maximal cystometric capacity rose from 104±68 ml to 116±107 ml (ns) and from 159±124 ml to 175±83 ml (ns), respectively. The decrease of the urinary frequency in the RTX group from 9.3±2 to 7.4±1.4 was also significantly greater (p=0.05) than that of the ethanol group, from 10.5±2 to 9.3±2 (ns).
RTX is safe for the bladder mucosa. Biopsies taken 1-2 years after one or more administrations did not reveal any changes (Silva et al.,'01). Therefore, taken together, our findings suggest that intravesical RTX is a promising therapy for neurogenic (spinal) and non neurogenic (idiopathic) forms of bladder overactivity. Similar findings in these diseases are being obtained by other groups in Italy (Lazzeri et al.,'97, Giannantoni et al.,'02) and Brasil (Palma et al.,01). In addition, RTX may also be useful in hypersensitive disorders of the bladder (Lazzeri et al.,'00). However, a more widespread use of RTX is limited at the moment by a shortage supply of the substance.