249

Burcher E[1], Liu L[1], Mansfield K J[1], Millard R J[2]
1. University of New South Wales, 2. Prince of Wales Hospital, University of New South Wales, Sydney, Australia

VANILLOID RECEPTOR EXPRESSION IN BODY AND TRIGONE OF FEMALE URINARY BLADDER: CHANGES IN SENSORY URGENCY

Hypothesis / aims of study

The vanilloid receptor (VR1) operates as a molecular integrator of noxious stimuli in peripheral terminals of sensory neurons. It is a nonselective cation channel activated not only by vanilloids such as capsaicin and resiniferatoxin, but also by protons (low pH), noxious heat and protein kinase C. Vanilloids selectively act on a subset of primary afferent nociceptive neurons and these agents have been used to treat incontinence resulting from neuropathic detrusor overactivity (1) and idiopathic detrusor overactivity (2). It has been hypothesised that vanilloid-sensitive neurons play an important role in the control of the micturition reflex. However, VR1 are also found on the bladder urothelium (3) and these may also be involved in the physiology or pathophysiology of micturition.

To date, there is no scientific basis for treatment of overactive detrusor with vanilloids. Therefore, the aims of this study were to establish a quantitative competitive RT-PCR technique to measure VR1 mRNA expression in the human urinary bladder, and to examine whether changes in VR1 mRNA expression occur in patients with sensory urgency (SU), a disorder characterised by small functional bladder capacity and by pain or discomfort.

Study design, materials and methods

Bladder biopsy samples were collected at cystoscopy from female patients, age range 18-78 years. Biopsies from body and trigone were obtained from patients with SU. All patients had undergone videourodynamics to exclude detrusor overactivity and to define first sensation to void. Age-matched patients with previous history of carcinoma acted as controls. Biopsies were collected into “RNA Later” and stored at 4°C overnight. Specimens were dissected into two layers, mucosa (epithelium and lamina propria) and detrusor muscle, before RNA extraction.

To construct the competitor RNA (internal deleted standard RNA, idRNA), a human VR1 receptor cDNA fragment (390 bp) was internally deleted ~20% using a restriction enzyme, Pst I. This modified internally deleted (id) cDNA was subcloned into pTargetT vector and used as a template to synthesize idRNA by T7 RNA polymerase. Total RNA (100 ng) and idRNA (variable amounts, 1-300fg) were reverse-transcribed (RT) with random hexamer and AMV reverse transcriptase, and the RT products were subsequently amplified by PCR with Tfl polymerase and a pair of VR1 gene specific primers. The RT-PCR products were then separated by gel electrophoresis (Fig 1A) and quantified by densitometry. For determination of the competition equivalence point, the data were analysed by linear regression (Fig 1B).

Results

RT-PCR amplified products corresponding to VR1 mRNA transcript and idRNA gave the expected 392 and 311 bp bands (Fig 1A). RNA RT-PCR determination of β-actin gene expression was used as an internal control to monitor the quantity and quality of total RNA of each sample. There were no significant differences in β-actin expression among groups (P = 0.86, one-way ANOVA).

In control biopsies, VR1 mRNA was present in both detrusor muscle (12.4 $\pm$ 3.2 fg idRNA) and mucosa (34.8 $\pm$ 8.5 fg idRNA) with a significantly higher expression in mucosa (n = 13, P = 0.007, paired t-test). In SU patients, the expression of VR1 was denser in mucosa from the trigone (41.3 $\pm$ 5.7 fg idRNA, n = 16) than in mucosa from the bladder body (18.3 $\pm$ 3.3 fg idRNA, n = 15, P = 0.002, unpaired t-test, Fig 2A). There was no significant change in VR1 expression in body mucosa from SU specimens compared with age–matched controls (both n = 15, P = 0.31, unpaired t-test). The expression level of VR1 mRNA in trigone mucosa was significantly inversely correlated with first desire to void (r^2 = 0.60, P = 0.002, Fig 2B). However, there was no correlation between VR1 expression in the mucosa of the bladder body from SU patients and their urodynamic data.

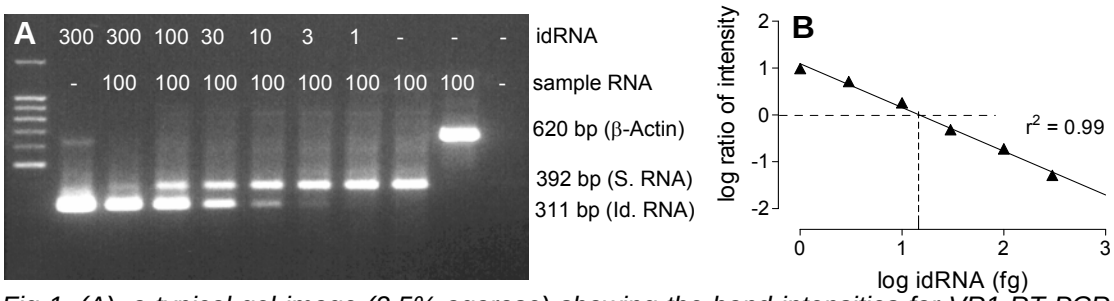

Fig 1. (A), a typical gel image (2.5% agarose) showing the band intensities for VR1 RT-PCR products obtained from bladder sample total RNA and idRNA. (B), quantitative analysis of Fig 1A. When the log ratio is zero, products from sample RNA and idRNA are equal; the amount of idRNA at this point represents the amount of VR1 mRNA in the sample (here, 15 fg idRNA/100 ng total RNA).

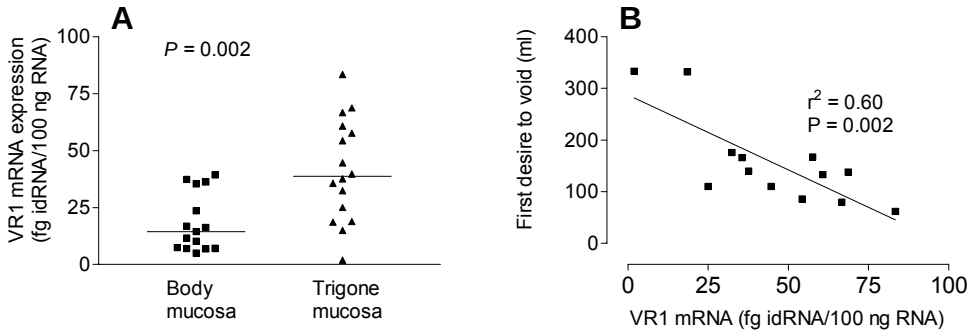

Fig 2. (A), in SU patients, expression of VR1 mRNA was significantly denser in trigone mucosa compared to body mucosa. (B), the amount of VR1 mRNA in trigone mucosa showed good correlation with first desire to void.

Interpretation of results

QC RT-PCR is a reliable technique for the quantification of VR1 mRNA in bladder detrusor and mucosa. The study showed a greater density of VR1 in mucosa compared with detrusor, in control patients. This study does not reveal the location of the "mucosal" VR1 which could be expressed on suburothelial sensory nerves, on the urothelium, or on both regions.

The trigone is an area associated with inflammation and pain in some SU patients. It was notable that there was no difference between control and SU patients in VR1 expression in the bladder body mucosa, whereas there was a significant elevation in VR1 in the trigone mucosa of SU patients.

Concluding message

These preliminary data show a higher expression of VR1 in the mucosa compared with the muscle of control patients. In SU patients, there was a higher expression in the trigone (mucosa) compared with the body (mucosa). The level of VR1 expression in the trigone was negatively correlated with the urodynamic parameter, first desire to void. The trigone is embyologically different from the bladder body and we hypothesise that excessive afferent signalling from this region may be related to an early first desire to void.

References

1. Intravesical treatment of overactive bladder. Urology 55: Suppl 5A, 60-64, 2000.
2. The effect of intravesical resiniferatoxin in patients with idiopathic detrusor instability suggests that involuntary detrusor contractions are triggered by C-fibre input. J Urol 168: 575-579, 2002.
3. Vanilloid receptor expression suggests a sensory role for urinary bladder epithelial cells. Proc Nat Acad Sci USA 98: 13396-13401, 2001.

FUNDING: Australasian Urological Foundation