

EXPRESSION OF CONNEXINS AND NEUROCHEMICAL RECEPTORS IN HUMAN DETRUSOR: DIFFERENCES IN GENDER AND IN MULTIPLE SCLEROSIS (MS)

Hypothesis / aims of study

Multiple sclerosis (MS) is an inflammatory demyelinating disorder, with females affected more than males [1]. Bladder dysfunction occurs in >80% patients with MS, and symptoms are related to lesions in the spinal cord as well as in the brain [2]. Neurogenic detrusor overactivity is found in the majority of patients: symptoms include hesitancy, urgency, frequency, nocturia and urge incontinence. Increased detrusor pressure, detrusor-sphincter dyssynergia (DSD), incomplete emptying, as well as urinary tract infections, are common. We hypothesized that MS was associated with changes in gene expression for muscarinic, tachykinin, purinergic and/or vanilloid receptors and for connexins (Cx) in detrusor muscle, and that expression was associated with clinical symptoms. Our aim was to quantify the mRNA expression of these genes in detrusor muscle: (a) in control males and control females, and (b) in MS, compared with control.

Study design, materials and methods

Bladder biopsies were collected into RNALater, and left at 4°C overnight. Specimens were dissected into detrusor and mucosa, and stored at -80°C. RNA was extracted from the detrusor samples and purified. cDNA was synthesized with SuperScriptTMIII RT Kits, and products were amplified with RealMasterMix Kits using real time PCR.

Data were analysed with respect to several housekeeping genes: β -actin, GAPDH and calponin, and to the calibrator sample (pooled control detrusor samples). MS patients comprised 10 males and 13 females, with median age 54 years. Patient symptoms and urodynamic findings were as follows: frequency/urgency/nocturia n=20, urge incontinence n=12, recurrent UTI n=11, low compliance n=5, phasic overactivity n=9. Asymptomatic control patients (35 males and 27 females, median age 59 years) were under investigation for haematuria or carcinoma surveillance. This study was approved by the local human ethics committees.

Expression of genes of interest relative to the internal standard calponin (muscle marker) was calculated using the standard formula $2^{-\Delta\Delta Ct}$ and expressed as fold-change. Data were expressed as median (IQR) and were compared using the Mann-Whitney test.

Results

Initial studies investigated whether the three housekeeping genes were uniformly expressed relative to gender, age and disease. GAPDH showed significant differences between male and female detrusor. β -actin results were even more variable. Calponin showed no difference in expression between gender or between control and disease, and it was chosen as the housekeeping gene for this study.

In control detrusor, differences in expression of several genes of interest were observed with respect to gender. The expression of muscarinic M2 and M3 receptors ($P<0.01$) and purinergic P2X1 receptors ($P<0.05$) mRNA was lower in control males ($n=29$) than in control females ($n=27$). There were no significant gender differences seen for tachykinin NK2 receptors, vanilloid TRPV1 receptors or connexins Cx43 and Cx45. No significant difference in expression with respect to patient age was observed for any of the seven genes tested.

In MS detrusor, gender differences were seen only for Cx45 and TRPV1 – in both cases, expression in females was significantly lower than in males ($P<0.05$).

Differences in expression for several genes were seen in MS. In females with MS, there was a decreased expression of mRNA for M3 receptors and Cx45 ($n=13$) compared with control female detrusor ($n=27$) (Figure 1). In contrast, males with MS showed no significant changes or even trends, compared with male controls. For all MS patients, expression of Cx45 was lower ($P<0.01$) in MS ($n=23$) than in control detrusor ($n=56$).

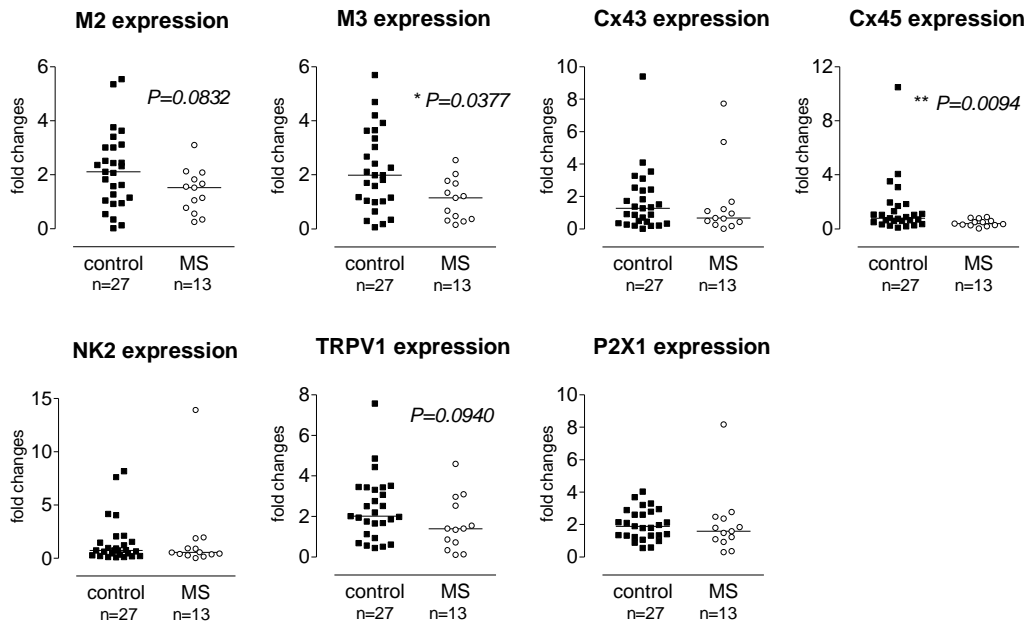


Figure 1. Expression of genes for neurochemicals and connexins, in detrusor of female control and MS patients. Data are expressed as fold change relative to the housekeeping gene calponin. Significant decreases in expression in MS were seen for muscarinic M3 receptors and for connexin Cx45. Trends for decreased expression were seen for muscarinic M2 and vanilloid TRPV1 receptors. *, $P < 0.05$; **, $P < 0.01$ (Mann-Whitney test)

MS patients were also grouped according to symptoms and urodynamic findings. The triad of frequency, urgency and nocturia (n=20) was associated with a significant ($P < 0.05$) decrease in detrusor M3 and TRPV1 receptors (females) and in Cx45 (both genders). A similar finding was made for urge incontinence (n=12). Recurrent UTI (n=11) was associated with significant decreases in several genes: M2 and M3 receptors ($P < 0.05$, females only) and in NK2 receptors ($P < 0.05$) and Cx45 ($P < 0.01$, both genders). Phasic overactivity (n=9) was associated with a decrease in Cx45 and in NK2 receptors ($P < 0.05$). Low compliance (n=5) was associated only with a significant decrease in Cx45 ($P < 0.05$).

Interpretation of results

With G-protein coupled receptor antibodies proving unsatisfactory for delineating receptors in human tissues, expression of mRNA is a reliable and highly specific technique to measure gene expression. However the question should be posed: do we expect to see changes in detrusor mRNA years after the progression of disease?

The gender differences observed in control detrusor may be related to the higher pressures seen in the male bladder, with down-regulation of muscarinic M2 and M3 receptors, and purinergic P2X1 receptors, which are all associated with detrusor contraction. Other gender differences were seen in MS detrusor. There have been sparse, rather variable reports of gender differences in muscarinic receptor mRNA in human bladder [3].

This appears to be the first study describing expression for these neurochemicals and connexins in bladder of MS patients. The major finding with respect to MS was the down-regulation of Cx45, a gap junction protein. This may be related to a deficiency of electrical transmission in the detrusor of MS patients. Of interest was the association of different symptoms and urodynamic findings with changes in different genes.

Concluding message

In control detrusor, females had greater expression of M3 and P2X1 receptors in detrusor, compared with age-matched males. The major difference between MS and control detrusor was a decreased expression of the connexin Cx45 in MS.

References

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Was the Declaration of Helsinki followed?	Yes
Was informed consent obtained from the patients?	Yes

