

DIFFERENTIAL REGIONAL EXPRESSION OF CONNEXIN ISOTYPES IN BLADDER SMOOTH MUSCLE

Hypothesis / aims of study

In the bladder, the presence of several connexin proteins (Cxs, the structural components of gap junctions) has been previously described. However the exact expression and distribution pattern of specific Cxs in the bladder is still unclear. Since each Cx forming-gap junction has specific channel permeability and gating properties, the connexin expression and distribution pattern in the bladder may have a significant impact on the functional demands of the bladder. The purpose of this study was to expand our understanding on the contribution of Cx43 and Cx45 in rat bladder tissue, with particular focus on quantifying the extent of expression of these gap junction proteins in different regions of the organ.

Study design, materials and methods

Bladder tissue was obtained from adult male Sprague Dawley rats. Urinary bladders were opened and the mucosa (urothelium and lamina propria) was carefully separated. Bladders were divided by micro-dissection in three pieces corresponding respectively to the base (from the neck to the trigone), the body (middle third of bladder) and the dome (upper third) of the bladder. From each segment were isolated the total RNA as well as protein lysate from the membrane fraction, and Cx45 and Cx43 gene and protein expression were investigated respectively by quantitative real-time rt-PCR and western blotting. In addition full length sections of bladder tissues (from base to dome) in which the urothelium was not removed were processed for immunofluorescence studies to detect changes in the distribution of Cx45 and Cx43 within the different regions of the bladder.

Results

Both Cx45 and Cx43 were confirmed to be extensively expressed in bladder tissue. Cx43 immuno-detection was more intense and diffuse than Cx45. Both Cxs were detected within the bundles of muscle showing a membrane-bound pattern around the smooth muscle cells, while in the mucosa, immuno-reactivity of both Cxs was more sparse. Real-time rtPCR showed Cx43 mRNA level significantly higher in the urothelium compared with the smooth muscle layers, in which the expression pattern was comparable between the base, body and dome of the bladder. However both western blotting and immunofluorescence analysis showed a significant increase in Cx43 expression in the dome of the bladder compared to the base and the body. In addition, the fluorescence intensity detected in the mucosa confirmed the increased pattern for Cx43 with more intense expression within the regions corresponding with the dome, compared with the mucosal areas of the base and body. Cx45 mRNA expression was significantly higher in the dome and in the body compared with base and mucosa. Western blotting analysis confirmed the increased expression in the dome but not in the body while immunofluorescence analysis showed that Cx45 was uniformly expressed within the body, the base and the dome of the bladder as well as the mucosa.

Interpretation of results

Connexin protein and gene expression confirms previous reports that rat bladder smooth muscle gap junctions are comprised predominately of Cx43 and Cx45. In particular Cx43 mRNA and protein appeared to be differentially expressed and localized in anatomically distinct areas of the bladder wall and mucosa. In contrast, the differential expression of Cx45 appears to be limited only to the transcriptional level while the protein distribution is not significantly different through the base, body or dome. The increased expression of Cx43 in the dome and in the mucosa of the bladder indicate that connexin proteins are differentially regulated in specific areas of the bladder, likely reflecting regional responses to particular physiological demands of the organ.

Concluding message

These findings taken together with the emerging concept that alteration of connexin expression is associated with several bladder dysfunctions can provide an understanding not only the physiological role of specific Cx45 and Cx43 in the bladder, but also whether changes in their relative ratio may occur in pathological conditions and thus lead to bladder dysfunction.

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