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INHIBITION OF GAP JUNCTION CHANNELS ALTERS BLADDER CONTRACTILITY

Hypothesis / aims of study

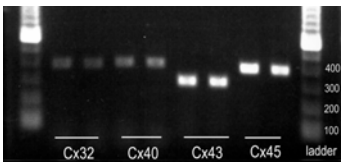
Several studies now indicate that connexins are present in both human and rat bladder, suggesting that, like other smooth muscle systems, the bladder may contain specialized structures enabling coupling between adjacent smooth muscle cells. Gap junctions are formed by various connexins (Cx), each belonging to a family of related proteins with distinct functional attributes. Thus, the effect of gap junctions on bladder contractility may depend on the properties of the specific connexin(s) identified in the bladder. Previous studies suggest that several different connexins are present in bladder tissue. However, the potential contribution of a specific connexin to bladder function has not been previously investigated.

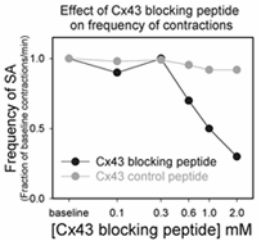
Study design, materials and methods

To confirm the expression of several connexin mRNA and proteins, and to localize specific connexins to structural gap junctions, Rt-PCR, Western blotting and immunogold labelling were performed, respectively. In vitro studies were conducted to investigate the functional contributions of gap junctions formed by Cx43 and Cx45. Blocking peptides were synthesized with sequences homologous to the extracellular loop of Cx43 and Cx45. These peptides interfere with the docking regions of connexins and thus selectively inhibit gap junction channels. Longitudinal strips of rat bladder tissue were suspended in temperature-controlled organ baths and placed under 2 grams of force. Bladder tissue was then exposed to increasing concentrations of the blocking peptides, individually or in combination. The amplitude and frequency of spontaneous activity were determined before and after administration of connexin blocking peptides.

Results

Amplification of cDNA from the bladder body yielded appropriately sized PCR products for Cx32, Cx40, Cx43 and Cx45. Expression of Cx40, Cx43 and Cx45 proteins were demonstrated by Western blot. Immunohistochemistry revealed Cx43 and Cx45 staining of bladder smooth muscle, while Cx40 staining appeared to be limited to intramural blood vessels. Immunogold electron microscopy showed Cx43 and Cx45 immunoreactivity organized in morphologically defined gap junctions between bladder smooth muscle cells. Cx43 blocking peptide attenuated the frequency of spontaneous contractions in a dose-dependent manner, but increased contraction amplitude. Administration of a synthetic peptide homologous to the intracellular loop of connexin43 had no effect on spontaneous activity. The combination of both Cx43 and Cx45 blocking peptides resulted in a marked reduction in both the amplitude and frequency of spontaneous activity.





Interpretation of results

Our findings suggest that functional gap junctions in rat bladder smooth muscle are formed primarily by Cx43 and Cx45 proteins. These connexins are two of the major constituents of gap junctions in vascular smooth muscle, although their distribution varies greatly throughout the vasculature. Alterations in spontaneous bladder activity induced by connexin blocking peptides indicate that both connexins may differentially participate in modulating bladder activity.

Concluding message

Since gap junctions play a role in modulating bladder smooth muscle function, pathological changes in the activity or density of these channels, or in the relative expression of constituent connexins may contribute to various types of bladder dysfunction.

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