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MEDIATORS OF HYPERTROPHY ALTER EXPRESSION OF CONNEXIN

Hypothesis / aims of study

Gap junctions are intercellular contacts connecting the surfaces of adjacent cells and thus promote cell-cell communication. In bladder tissue, the presence of several connexins (Cx, the structural component of gap junctions) has been described. However the exact expression pattern, distribution and possible roles of these structures are still unclear. Recent studies showed that particular bladder dysfunctions (i.e. bladder outlet obstruction), were associated with alteration in Cxs expression[1,2], however it is not completely clear which Cxs are involved and the direction of these changes. In the present study, we investigated Cx37, Cx40, Cx43, and Cx45 gene and protein expression in response to mediators of hypertrophic processes such as continuous Angiotensin II (AngII) and mechanical activation, and we investigated whether changes in Cxs are coupled with altered bladder contractility.

Study design, materials and methods

RT-PCR, Western blotting and immunofluorescence were performed to detect the Cx37, Cx40, Cx43, Cx45 gene and protein expression and localization in rat bladder tissue. *Functional studies:* After removal of the urothelium, longitudinal strips of rat bladder were stretched to 1.5 grams of force in organ baths. The contractile responses induced by continuous electrical field stimulation (EFS, 20-30V, 0.1Hz, 0.5ms) or AngII (1 μM) were recorded for 8 hours. Separate bladder strips were incubated with an AngII receptor antagonist (losartan, Los,10 μM) and stimulated for 8 hours. Amplitude of induced contractions was continuously recorded. After 8 hours, total RNA was isolated and real-time PCR was performed to determine the expression of Cx37, Cx40, Cx43, and Cx45 genes.

Results

RT-PCR and Western blotting analysis confirmed the expression of all Cxs investigated. Immunofluorescence imaging with double labeling showed Cx45, and Cx43 localized in bundles of smooth muscle, while Cx37 and Cx40 were more likely expressed on intramural blood vessels. Both AngII and EFS induced a significant up-regulation of Cx37, Cx40, Cx43, and Cx45 gene and protein expression in urinary bladder smooth muscle after 8 hours of continuous stimulation. No differences were found in control, non-stimulated samples. Continuous EFS caused a gradual and significant increase in contraction amplitude for 8 hours. Moreover Losartan significantly attenuated the up-regulation of all Cx genes caused by 8 hours of continuous stimulation and in parallel decreased the force generated by EFS.

Interpretation of results

These findings suggest that adaptive responses to physiologic stress induced by continuous smooth muscle stimulation and AngII receptor activation result from increases in Cx37, Cx40, Cx43 and Cx45 gene and protein expression. Furthermore, the prevention of connexin upregulation by an AngII receptor antagonist Losartan suggests a possible role for a local renin-angiotensin system in regulating the expression of specific Cxs.

Concluding message

The coordination of the hypertrophic response of bladder smooth muscle to outlet obstruction may depend on cell-to-cell communication through gap junctions and appropriate regulation of connexin expression.

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