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ROLE OF CAVEOLAE IN THE MODULATION OF SPECIFIC RECEPTOR-MEDIATED SIGNAL TRANSDUCTION PATHWAYS IN RAT BLADDER

Hypothesis / aims of study

Caveolae are cholesterol-rich plasmalemmal microdomains that serve as sites for sequestration of signalling proteins and thus may facilitate, organize and integrate responses to extracellular stimuli. Although little attention has been focused on the functional significance of these organelles in bladder smooth muscle, recent evidence indicates that aging and pregnancy are associated with a reduction in caveolae in the bladder. These findings suggest that impaired detrusor contractility accompanying these physiologic processes may result from depletion of bladder smooth muscle caveolae.

Caveolin proteins are structural components of caveolae that function as scaffolding proteins and signal transduction regulators. Accordingly, deletion of caveolin-1 in knockout mice results in loss of caveolae in bladder smooth muscle. Although these animals exhibit marked bladder dysfunction, the pathways leading to these functional changes are unclear. The purpose of this study was to determine the role of caveolae in the modulation of receptor-mediated signal transduction.

Study design, materials and methods

Reverse transcriptase-polymerase chain reaction was performed to detect the expression of caveolin-1, caveolin-2, caveolin-3 mRNA in rat bladder tissue.

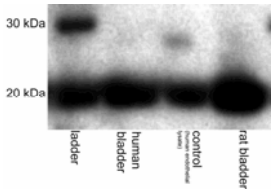
Immmunohistochemistry and Western blotting were performed to confirm the presence of caveolin proteins and localize these proteins in rat bladder tissue.

For in vitro studies, the urinary bladder was removed from male rats after sacrifice and placed in cold Kreb's solution. Longitudinal strips of bladder tissue were suspended in an organ bath maintained at 37ºC, placed under 1.5 grams of force and equilibrated for 45 min. Contractile responses to serotonin (10µM), angiotensin II (1µM), KCl (50mM) and carbachol (0.1µM-10µM) were determined. Tissue was exposed to methyl-ß-cyclodextrin (10-15 mM) for 1.5 hours to deplete membrane cholesterol and thus disrupt the integrity of caveolae. Responses to agonists were repeated after cholesterol depletion as well as after replenishment of cholesterol (5.1mM). Control responses to agonists were obtained from tissue that remained untreated.

Electron microscopy was performed to determine the morphologic effects of methyl-ß-cyclodextrin treatment. Bladder tissue was incubated in cyclodextrin, cholesterol or cyclodextrin followed by cholesterol, or left untreated and then fixed in glutaraldehyde and processed for electron microscopy.

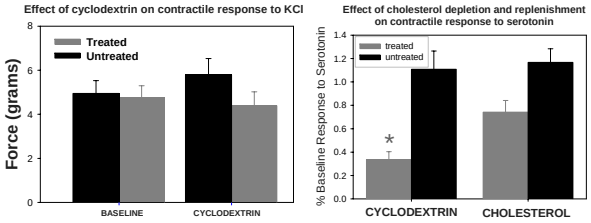
Results

Amplification of bladder cDNA yielded appropriately sized PCR products, corresponding to the expression of caveolin-1, caveolin-2, caveolin-3 mRNA. No PCR products were generated in samples in which reverse transcriptase was omitted. Western blot analysis (right) detected expression of caveolin-1 protein (22 kDa) in both human and rat bladder. Human endothelial lysate derived from cultured aortic endothelial cells was used as positive control. Caveolin-3 protein (18 kDa) expression was also detected in rat bladder. Rat striated muscle lysate was used as positive control.



Intense punctuate immunoreactivity was detected on the periphery of bladder smooth muscle cells indicating localization of caveolin-1 on the plasma membrane. Although caveolin-2 immunoreactivity was sparse in smooth muscle bundles, staining was consistent with localization in urothelial cells and suburothelial blood vessels. Caveolin-3 immunoreactivity was similar to that of caveolin-1, but less intense and more diffuse. Immunostaining was not detected in sections incubated without primary antibodies.

Contractile responses to KCl and spontaneous smooth muscle activity were not affected by cyclodextrin. In contrast, contractile responses to serotonin and angiotensin II were significantly attenuated by cyclodextrin. Addition of cholesterol to the baths restored



contractile responses towards baseline levels. The contractile response to carbachol was not affected by treatment with cyclodextrin.

In thin sections processed for electron microscopy, untreated bladder tissue showed abundant, typical flask-shaped caveolae in the plasma membrane. Conversely, in tissue treated with methyl-ß-cyclodextrin, plasma membrane invaginations were essentially absent, suggesting loss of structural caveolae. Morphologic structures indicative of caveolae were clearly visible in tissue in which cholesterol was restored.

Interpretation of results

Spontaneous activity and contractile responses to KCl were not affected by cyclodextrin treatment, suggesting that contractile mechanisms were not impaired by disruption of caveolae. Furthermore, cholesterol depletion reduced the contractile responses to serotonin and angiotensin II while cholesterol replenishment restored contractile responses, suggesting that caveolae are essential for these agonist induced responses. However, the lack of effect of cyclodextrin on contractile responses to carbachol indicates that signaling events initiated by muscarinic receptor activation are not sensitive to caveolae depletion.

Concluding message

Our study indicates that specific agonist-induced signaling pathways that regulate bladder smooth muscle contraction occur in specialized plasmalemmal microdomains and depend in part on the integrity of caveolae. Thus, pathologic disruption of caveolae or alterations in the number of membrane caveolae may significantly impair bladder contractility.

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