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INTERACTION AND REGULATION OF CAVEOLIN AND ANGIOTENSIN II IN BLADDER SMOOTH MUSCLE

Hypothesis / aims of study

Although many aspects of bladder contractility have been established, the exact mechanisms regulating specific agonists induced smooth muscle responses are still unclear. Angiotensin II (AngII), the effector protein of the renin-angiotensin system, has been found not only in the bladder[1], but also in other tissues of the upper and lower urinary tract (i.e. urethra, ureter, corpora cavernosa). Signaling processes induced by AngII have been implicated in the hypertrophic response in many smooth muscle systems but the role of this peptide in the bladder needs to be further elucidated. Previous studies have shown that bladder smooth muscle caveolae, cholesterol enriched plasmalemmal microdomains, are involved in the positive regulation of several agonist-induced signaling processes, including AngII[2]. Thus, the purpose of this study was to investigate the structural relationship between the AngII receptor and the caveolin proteins, and whether the activation of the AngII receptor (AT_1R) by continuous stimulation with AngII alters expression of caveolins.

Study design, materials and methods

Immuno-precipitation was carried out to demonstrate the interaction between caveolin protein and AT_1R. *Functional studies*: Urinary bladders were obtained from adult SD anesthetized male rats and the urothelium was carefully removed by micro-dissection. Longitudinal strips were processed for *in vitro* tensiometry studies. The amplitude and frequency of both spontaneous and AngII (1 μM) induced activity were recorded for 8 hours. In parallel, force was recorded in control strips similarly obtained from the same bladder that remained un-stimulated. *Gene and protein studies*: After 8 hours, total RNA and protein were isolated from each AngII stimulated and un-stimulated control strips. Quantitative real-time PCR was performed to determine the gene expression of Cav-1, Cav-2, and Cav-3, Western blotting and immunofluorescence techniques were performed to determine protein expression and distribution.

Results

AT_1 co-precipitated with both Cav-1 and Cav-3 proteins. Chronic AngII stimulation resulted in an augmented amplitude of the phasic activity at the end of the 8 hours compared to baseline (0 hrs). AngII (1 μM) induced a significant up-regulation of gene expression of caveolin-1 (6.6 ± 1.8 fold), caveolin-2 (8.4 ± 3.3 fold) and caveolin-3 (7.9 ± 3.0 fold) in urinary bladder smooth muscle after 8 hours of stimulation in comparison with control non-stimulated samples. In contrast, western blotting and immunofluorescence analysis showed a decrease in the level and distribution of cav-1 protein.

Interpretation of results

Contractile responses to AngII are mediated by the interaction of caveolin proteins with the AngII receptor. AngII induces an up-regulation of caveolin-1, 2, and 3 gene expression. The increased bladder smooth muscle contraction during 8 hours of AngII exposure is consistent with an enhanced positive regulation of AngII mediated signaling by caveolae. These findings may have important implications for pathophysiologic processes linked to alterations in AngII secretion such as the hypertrophic response to bladder outlet obstruction.

Concluding message

AngII-induced regulation of caveolin expression may play a role in modulating bladder smooth muscle function.

References

1. Adv Exp Med Biol. 462:183-91, 1999
2. Neurourol Urodyn. 26:71-80, 2007

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