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Cristofaro V[1], Golabek T[1], Radisavljevic Z M[1], Yalla S[1], Sullivan M[1]
1. *Boston VA Healthcare System, Harvard Medical School*

ANGIOTENSIN II MODULATES DETRUSOR CONTRACTION BY UP-REGULATING CAVEOLIN PROTEINS

Hypothesis / aims of study

Angiotensin II (AngII), the effector molecule of the renin-angiotensin system, is known to participate in smooth muscle remodelling during hypertrophy in several tissues including the bladder. Although autocrine AngII can be released from bladder smooth muscle (BSM) cells in response to mechanical stimulation [1], little is known about the mechanisms by which AngII-mediated signals are regulated in the bladder. Previous studies showed that contractile responses induced by AngII depend on the integrity of BSM caveolae [2], specific membrane invaginations involved in the regulation of signal transduction. Moreover, alterations in caveolar elements have been described in hypertrophic detrusor tissue from obstructed rat bladders [3], an animal model in which AngII upregulation has also been implicated. In this study, we investigated the molecular effects of AngII stimulation on the expression of caveolins (Cav, the structural proteins of caveolae), and whether changes in caveolins induced by AngII may be associated with functional alterations responsible for the development of bladder dysfunction.

Study design, materials and methods

Bladder tissue was procured from male adult Sprague Dawley rats, in which the mucosa was removed. The molecular interaction between AngII receptor-1 (AT1R) and Cav-1, Cav-2 and Cav-3 in BSM tissue was investigated by co-immunoprecipitation. For *in vitro* functional studies, longitudinal bladder strips were stretched under 1.5 grams of tension in organ bath at 37ºC, and equilibrated for 45 minutes. After equilibration, tissue strips were challenged for a period of 8 hours with continuous exposure to exogenous AngII, or with chronic electrical field stimulation (EFS, EFS, 20V, 0.1Hz, 0.5ms) in the presence or absence of AT1R antagonist Losartan. Control data was acquired from separate tissue strips under non-stimulated conditions. Changes in the amplitude of force generated in response to treatments were monitored during the entire period of stimulation. The effect of chronic EFS on the release of endogenous AngII was measured by enzymatic immunoassay. After stimulation, total RNA and protein were isolated from each strip of tissue and Cav-1, Cav-2 and Cav-3 gene and protein expression was determined by real-time rtPCR and western blotting respectively.

Results

AT1R co-precipitated with Cav-1, Cav-2 and Cav-3. Continuous exposure of BSM tissue to exogenous AngII resulted in a significant up-regulation of Cav-1, Cav-2 and Cav-3 gene and protein expression compared to non stimulated control tissue. Similarly Cav-1, Cav-2 and Cav-3 expression was up-regulated in BSM tissue after 8 hours of chronic EFS. Release of endogenous AngII was detected from electrically stimulated bladder tissue after 8 hours compared to non stimulated control tissue. Continuous exposure to AngII induced a progressive and significant increase in the amplitude of bladder spontaneous contractions compared to non stimulated tissue. Similarly, in electrically stimulated tissue the amplitude of EFS-induced bladder contractions was significantly augmented after a period of 8 hours. However, the increase in EFS-induced contractions was prevented by pre-incubation with AT1 receptor antagonist Losartan.

Interpretation of results

The molecular interaction of Cav-1, Cav-2 and Cav-3 isoforms with AT1R is consistent with the localization of this receptor in membrane caveolae and its regulation by caveolin proteins. Chronic perturbation of BSM, exemplified by continuous EFS, enhances contractility due to endogenous AngII-mediated activation of AT1 receptors. The up-regulation of caveolin proteins, caused by AngII (either exogenously delivered or endogenously released by EFS) and prevented by AT1R inhibition, suggests that AngII released by BSM initiates a positive feedback response in which AT1 receptor-activated detrusor contractions that are facilitated by caveolae become augmented by an enhanced caveolin-AT1R interaction.

Concluding message

These findings may have important implications for pathophysiologic detrusor processes associated with altered AngII secretion such as the hypertrophic response to bladder outlet obstruction.

References

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Disclosures

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