

## EFFECT OF AGE ON CAVEOLAE-MEDIATED SIGNALING PATHWAYS IN BLADDER SMOOTH MUSCLE.

### Hypothesis / aims of study

Aging in both men and women is associated with several types of bladder dysfunction. Although the possible causes of bladder abnormalities related to aging have been investigated, the exact mechanisms involved in these disorders remain unclear. A previous ultrastructural study of bladder biopsies from elderly patients described a decrease in the number of caveolae in smooth muscle cells [1]. These specialized membrane microdomains serve as platforms to modulate and integrate signalling components that are activated in response to various physiologic agonists. Alterations of these structures have been shown to result in abnormal regulation of specific receptor-activated signaling pathways [2]. In this study, we investigated whether caveolar elements become altered in the bladder during aging, and whether these alterations contribute to bladder dysfunction.

### Study design, materials and methods

Urinary bladders from Male Sprague Dawley rats at three different ages (10 weeks, 6 months and 12 months) were compared. The gene expression and protein distribution of caveolins (Cav, structural components of caveolae), were investigated by real time rtPCR and immunohistochemistry respectively. For functional studies, longitudinal bladder strips were obtained from each group. The urothelium was removed and tissue was stretched to 1.5 grams of force and mounted in organ bath maintained at 37°C. Contractile responses to serotonin (5-HT) and phenylephrine (PE) have been shown previously to be specifically sensitive to caveolar integrity, while carbachol induced signalling does not appear to be mediated by caveolae. Therefore, contractile responses induced by these agonists were measured before and after biochemical disruption of caveolae, achieved by methyl- $\beta$ -cyclodextrin (m $\beta$ cdx), as well as after caveolae were restored by exposure to soluble cholesterol.

### Results

Compared with bladders from 10 week old rats, the gene expression of Cav-3 was significantly decreased in older rats. Consistent with this finding, immunohistochemistry analysis identified a decrease in Cav-3 protein distribution in bladder tissue from aged rats relative to 10 week old rats. However, differences in Cav-1 and Cav-2 expression were not readily detected. The contractile response to 5-HT in the youngest group was significantly reduced after caveolae depletion, but was restored to baseline levels after caveolae reformation, consistent with the loss, and subsequent reestablishment, of the positive modulation imparted by caveolae. In contrast, in bladder strips from both 6 and 12 month old rats, neither the disruption of caveolae by m $\beta$ cdx nor their restoration by cholesterol altered the responses induced by 5-HT. Baseline responses to 5-HT were age dependently reduced compared with younger animals. In bladders from 10 week old rats, the contractile response induced by PE significantly increased after and was restored to baseline levels after cholesterol replenishment, confirming the negative regulation imparted by caveolae for this agonist. In bladders from 6 month old rats, the response to PE was not significantly affected by either caveolae disruption or reformation, and the amplitude of contraction at baseline was not significantly different compared with younger rats. In bladders from 12 month old rats however, the amplitude of PE was significantly higher at baseline compared with 10 week and 6 month old rats. Disruption of caveolae by m $\beta$ cdx did not significantly affect responses induced by PE in bladders from the 6 month old group, while in bladders from 12 month old rats the amplitude of PE induced responses was increased after m $\beta$ cdx. The contractile response induced by carbachol (Cch) was not different at baseline among the three groups. Neither m $\beta$ cdx nor cholesterol affected the amplitude of Cch responses from any group.

### Interpretation of results

The decrease in Cav-3 protein and gene expression in bladder from aged rats is consistent with a progressive reduction in caveolae-mediated signalling with aging. The age-dependent decrease of 5-HT contractile responses at baseline, together with the loss of sensitivity to caveolar depletion in bladder from older rats, suggest a progressive loss of the positive regulation of 5-HT which is normally imparted by intact caveolae in bladders from young rats. Furthermore, the markedly increased PE response at baseline seen in the aged bladder is consistent with a loss of negative regulation of caveolae-mediated signaling. In contrast, the lack of effect of m $\beta$ cdx on Cch induced responses in all three groups confirms previous reports showing that caveolae are unlikely involved in the modulation of signaling pathways initiated by this agonist in rat bladder tissue [2].

### Concluding message

Alteration of specific caveolae-mediated signaling pathways in bladder from aging rats suggests that aberrations in membrane caveolae or caveolin proteins in bladder smooth muscle cells may occur with aging, and thus contribute to the development of bladder dysfunction.

### References

1. Elbadawi A, Yalla SV, Resnick NM: Structural basis of geriatric voiding dysfunction. II. Aging detrusor: normal versus impaired contractility. *J Urol.* (1993);150:1657-67
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