

## ALTERATION OF CAVEOLAE-MEDIATED SIGNALLING PATHWAYS IN OBSTRUCTED BLADDER

### Hypothesis / aims of study

Bladder outlet obstruction (BOO) can lead to voiding dysfunction not only in response to the increased outlet resistance, but also as consequence of structural changes occurring in the bladder in response to obstruction. One of these changes may involve the integrity of caveolae, specialized plasmalemmal microdomains enriched in cholesterol, which have the important role to differentially potentiate or attenuate bladder smooth muscle responses to specific physiologic agonists [1]. Thus it is possible that alterations in caveolae may result in a dysregulation of these agonist-induced responses, and potentially contribute to the development of bladder dysfunction. The goal of this study was to identify changes in caveolae or caveolar elements that occur with bladder outlet obstruction, and determine whether these changes could potentially affect the functional responsiveness of the obstructed bladder.

### Study design, materials and methods

BOO was created in 10 week old Sprague Dawley male rats and maintained for a duration of 1, 2 or 4 weeks. A silk ligature was placed around the proximal urethra and tied around a 3F catheter that was positioned parallel to the urethra to create a standardized degree of obstruction. Control animals underwent to the same surgical procedure with the omission of the final ligature. Differences in the number and in the ultrastructure of bladder smooth muscle caveolae after BOO were investigated using electron microscopy. Changes in caveolin protein expression and distribution after BOO were determined by real time rt-PCR and immunofluorescence microscopy. For functional studies longitudinal bladder strips from control and obstructed bladders were stretched in organ bath at 37°C and processed for *in vitro* tensiometry. The contractile responses induced by physiologic agonists were repeated before and after disruption of caveolae which was achieved by methyl- $\beta$ -cyclodextrin (m $\beta$ cd), and compared with non-obstructed controls.

### Results

After 2 weeks of BOO, the bladder weight markedly increased compared to non-obstructed controls. The number of caveolae significantly decreased after 2 weeks of BOO compared with non-obstructed bladders. Cav-1 immunoreactivity diminished after 1 week of BOO and further decreased after 2 weeks of BOO. Cav-2 immunoreactivity decreased after 1 week of BOO but was less attenuated after 2-weeks. Cav-2 gene expression was significantly up-regulated after 2 weeks of BOO. Following 2 wks of BOO, the contractile response to bradykinin (BK) did not change after caveolar depletion compared with non-obstructed animals, in which m $\beta$ cd significantly increased the BK-induced contraction; however in BOO animals the amplitude of BK induced contraction was greater at baseline compared with non-obstructed controls. In non-obstructed bladders, the contractile response to serotonin (5-HT) was diminished after caveolar depletion. In BOO, the 5-HT induced response was only slightly diminished by m $\beta$ cd after 2 weeks, while after 4 weeks the contractile response was completely unaffected by m $\beta$ cd.

### Interpretation of results

This study demonstrated that the structural loss of caveolae with BOO is associated with functional alteration of specific agonist-induced responses that require intact caveolae. These data are consistent with a reduction in the negative and positive modulation of BK and 5HT induced contractions respectively, which in non-obstructed bladders is normally imparted by caveolae. This caveolae-mediated modulation is progressively lost during bladder obstruction as these structures gradually diminish from the bladder smooth muscle membrane.

### Concluding message

Alterations in membrane caveolae and caveolin protein expression in obstructed bladder may play a role in the development of bladder dysfunction induced by BOO due to impaired caveolae-mediated modulation of specific receptor-activated signaling pathways.

### References

1. V. Cristofaro, C.A. Peters, S.V. Yalla and, M.P. Sullivan: Smooth Muscle Caveolae Differentially Regulate Specific Agonist Induced Bladder Contractions. *Neurourology and Urodynamics*. (2007);26(1):71-80

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