

CHANGES IN CAVEOLAE MEDIATED PURINERGIC-MUSCARINIC TRANSMISSION IN CYSTITIS

Hypothesis / aims of study

Interest in the plausible involvement of caveolae, omega shaped membrane invaginations, in bladder signal transduction during health and disease has emerged during the past years.

The corner stone in the bladder “caveolae signal transduction theory” is the expression and/or translocation of receptors important for bladder signalling, such as muscarinic receptors and purinoceptors, to caveolae. Studies regarding caveolae and inflammatory bladder disorders, such as bladder pain syndrome/interstitial cystitis (BPS/IC), are sparse in number. However there are studies showing the caveolae specific protein caveolin-1 to be reduced during bladder inflammation, suggesting a change in caveolae-dependent signalling during cystitis (1).

A deeper understanding of the caveolar involvement in bladder signalling is of importance in order to fully unravel the mechanisms of micturition and progression of inflammatory bladder diseases. This project aims to further investigate the functional role of the caveolae in purinergic and muscarinic receptor signalling, in both normal and inflammatory conditions, via depletion of caveolar cholesterol structures.

Study design, materials and methods

The current study was performed using organ bath preparations; both the classic full thickness bladder strip preparation and a whole bladder preparation. In the whole bladder preparation two catheters were inserted through the inner urethral orifice and ligated to secure that the only in and outflow were via the two catheters. The rats were either healthy controls or pre-treated with cyclophosphamide (CYP; 100 mg/kg, 60 hours prior to the experiment) to induce bladder inflammation.

Sprague Dawley rats (400 – 700 g) were euthanized using an overdose of pentobarbitone potassium and a subsequent cut through the heart. The excised bladders were used for full thickness bladder strip preparation or mounted whole, as described above, on a fixed steel rod and coupled to a force transducer immersed into an organ bath system filled with Krebs solution. Increasing concentrations of methacholine (10^{-8} M – 10^{-3} M) and adenosine-tri-phosphate (ATP; 10^{-6} M – 10^{-3} M) were added cumulatively before and after caveolae depletion using methyl- β -cyclodextrin (Me- β -CD; 10 mM, 1 hour). Electrical field stimulation (EFS; 2Hz – 60 Hz) was also employed for bladder strip preparations.

Results

Depletion of the caveolar cholesterol structures in the bladder strip preparations showed a statistically significant decrease in response to purinergic stimulation for control rats (from a maximal contraction, at 10^{-3} M, of 1.78 ± 0.42 to 0.59 ± 0.38 mN; $n=6$; $p < 0.05$). A trend towards the same change was strongly indicated in the control whole bladder preparations as well, however not significant (from a maximal contraction, at 10^{-3} M, of 7.45 ± 2.14 to 4.01 ± 1.40 mN; $n=7$; not significant). The purinergic stimulation of preparations from CYP-pre-treated animals was not significantly affected in any of the preparation methods (from a maximal contraction, at 10^{-3} M, of 3.02 ± 1.38 to 1.43 ± 0.72 mN and from 6.76 ± 1.34 to 4.62 ± 1.10 mN for full thickness bladder strips and whole bladder preparations respectively; $n=5$ for both groups).

Interestingly, contraction to EFS was significantly decreased in control bladder strip preparations after treatment with Me- β -CD (from a maximal contraction, at 40 Hz, of 10.54 ± 2.81 to 1.58 ± 1.09 mN; $n=7$; $p < 0.001$), this decrease was not observed for CYP-pre-treated animals. Furthermore, no significant decrease in maximal contraction, evoked by administration of Krebs solution containing a high concentration of potassium (124 mM; sodium exchanged for potassium) was observed after treatment with Me- β -CD for control rats (from a maximal contraction of 14.52 ± 3.62 to 10.44 ± 3.84 mN; $n=10$; not significant).

The contractile muscarinic response, in both full thickness bladder strips and whole bladder preparations, did not seem to be affected by depletion of the caveolae cholesterol structures in neither control nor CYP-pre-treated animals. For full thickness bladder strip preparations the maximal contraction to methacholine, at 10^{-3} M, was 12.69 ± 3.04 and 10.57 ± 2.70 mN for controls ($n=10$) and 14.98 ± 2.69 and 14.00 ± 2.34 mN for CYP-pre-treated ($n=5$) rats before and after depletion of caveolae, respectively. For whole bladder preparations the maximal contraction to methacholine, at 10^{-3} M, was 16.33 ± 2.56 and 14.26 ± 2.10 mN for controls ($n=12$) and 10.07 ± 4.17 and 14.10 ± 6.69 mN for CYP-pre-treated ($n=8$) rats before and after depletion of caveolae, respectively.

Interpretation of results

In concordance with previous studies these preliminary results showed no significant changes in muscarinic contraction following caveolar cholesterol depletion regardless of bladder preparation method (whole bladder- or full thickness bladder strip preparation). The same applies in the case of direct depolarisation of the detrusor muscle by high concentrations of potassium, which also remained relatively unchanged. Interestingly, the contractile responses to both ATP and EFS were significantly decreased after Me- β -CD treatment in control strip preparations. During inflammation, however, such differences were not present. Previous studies have shown a decrease of caveole specific protein during inflammation, which may shed some light on the present findings regarding CYP-induced cystitis. Likely, the importance of caveolar signalling is reduced during cystitis which explains the lack of change in contractile response after Me- β -CD administration in inflamed bladders. This may, in turn, be compensated for by for instance upregulation of functional cholinergic M3 receptors. In the normal case on the other hand, the caveolae seem to be important for neuronally evoked bladder contractions as well as for contractile responses to ATP. Thus, the latter is likely of great interest as an intermediary signalling step involved in the release of other, perhaps cholinergic, transmitters responsible for exerting the direct smooth muscle contraction.

Concluding message

Depletion of caveolar cholesterol structures by Me- β -CD-treatment significantly decreases the purinergic response in healthy rat urinary bladders. This is mirrored by a significant decrease in neuronally evoked contraction to EFS in control rats. Preparations from CYP-pre-treated animals were not affected by the depletion of caveolae. An intermediary signalling step, involving ATP and being largely caveolae-dependent, may be hampered by inflammatory processes. Consequently, this can be one pathophysiological mechanism behind diseases like interstitial cystitis.

References

1. Kim SO, Song SH, Lee SC, Cho KA, Yu HS, Hwang IS, et al. (2012). Expression of caveolin-1 in rat urinary bladder with cyclophosphamide-induced cystitis. International neurourology journal 16(4): 169-174.

Disclosures

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