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LOSS OF DETRUSOR SMOOTH MUSCLE CAVEOLAE ALTERS ALPHA-1 ADRENERGIC SIGNALING IN OVERACTIVE BLADDERS FROM SPONTANEOUSLY HYPERTENSIVE RATS

Hypothesis / aims of study

Caveolae, flask-shaped invaginations on the plasma membrane enriched in cholesterol and sphingolipids, are organized microdomains that play a crucial role in regulating specific receptor−activated signaling events in a variety of cell types, including bladder smooth muscle (BSM) [1]. Compared to control animals, the density of caveolae in BSM cells is significantly reduced in spontaneously hypertensive rats (SHR), an animal model of detrusor overactivity (DO), suggesting that the integrity of these structures is important for normal detrusor function [2]. Although the etiology of DO remains unresolved, alterations in adrenergically-mediated signaling have been proposed in its pathogenesis [3], since α-adrenoceptor (AR) expression is increased in overactive bladders and non-selective α1AR antagonists relieve irritative symptoms in both men and women. Our previous study showed that α−AR-mediated contractions in BSM are sensitive to depletion of caveolae [1]. The purpose of this study was to investigate whether the intrinsic loss of caveolae in SHR bladders might contribute to the development of DO by dysregulation of α1−AR activated pathways.

Study design, materials and methods

Overactive bladders were procured from SHR rats, while Wistar Kyoto (WKY) rat bladders were used as control. For functional studies, longitudinal bladder strips were mounted in organ bath containing Kreb's Solution at 37°C and stretched under 1.5 grams of tension. Adrenergically-mediated bladder contractions were induced by administration of phenylephrine (PE), a non-selective α1-adrenoceptor (AR) agonist, added to the bath either alone or in the presence of a selective α1A-AR antagonist SNAP or α1D-AR antagonist BMY. PE responses were repeated after the administration of methyl-β-cyclodextrin (mβCD), an agent that disrupts the integrity of caveolae by depleting membrane cholesterol. PE responses were repeated after incubation with water-soluble cholesterol, to restore the integrity of caveolae by replacing membrane cholesterol. Western blotting was performed to detect expression of α1A– and α1D-AR in WKY and SHR BSM tissues. The molecular interaction between α-AR subtypes and caveolin proteins in both WKY and SHR bladders were investigated by co-immunoprecipitation (Co-IP) experiments.

Results

The amplitude of PE-induced contractile responses at baseline was significantly higher in SHR bladders compared to WKY. Moreover, in WKY, the experimentally-induced depletion of caveolae achieved by mβCD significantly increased the PE responses, while the restoration of caveolae integrity by cholesterol replenishment re-established responses toward baseline levels. In contrast, neither the administration of mβCD nor the replenishment of cholesterol significantly affected PE responses in SHR. No differences in α1A-AR or α1D-AR expression were detected by Western blot between WKY and SHR bladders. Both α1A-AR antagonist SNAP and α1D-AR antagonist BMY significantly decreased baseline contractile responses to PE to a comparable extent between WKY and SHR bladders. In the presence of SNAP, neither the depletion of caveolae nor their reformation by cholesterol replenishment had a significant effect on PE responses in either WKY or SHR. In contrast, the administration of mβCD in the presence of BMY significantly increased the contractile response to PE in both strains, an effect that was significantly more pronounced in WKY. α1A-AR immunoprecipitated with Cav-3 but not with Cav-1 in BMS tissue from both WKY and SHR.

Interpretation of results

The augmented responsiveness to PE in SHR bladders compared to WKY, together with the unchanged α1-AR expression between strains suggest a loss of inhibition on adrenergic-mediated responses in SHR caused by the reduced number of caveolae on BMS from this strain. The increase in contractile responses to α1A−AR activation after administration of mβCD and the molecular interaction of α1A−AR with Cav-3 suggest that caveolae impart a negative regulation of signaling induced by this AR subtype. In contrast, responses mediated by α1D−AR do not appear to be dependent on caveolar integrity.

Concluding message

These findings support the importance of caveolae in modulating specific signaling processes in the bladder, and demonstrate that loss of integrity of these microdomains results in significant alteration of bladder responsiveness to adrenoceptor-activated signaling that could potentially contribute to the development of DO.

References

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