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MUSCARINIC RECEPTOR SUBTYPES ARE INVERSELY REGULATED BY BLADDER SMOOTH MUSCLE CAVEOLAE

Hypothesis / aims of study

Caveolae are specialized regions of the cell membrane that provide a structural platform to organize and compartmentalize various signalling molecules. These cholesterol enriched membrane domains thus serve to modulate and integrate a number of cell-surface signal transduction events [1]. In the bladder, caveolae regulate specific receptor-activated signaling pathways, by potentiating or attenuating contractile responses in an agonist-dependent manner [2]. However, contractile responses to cholinergic agonists in rats are not affected by loss of membrane caveolae. Nevertheless, whether specific muscarinic acetylcholine receptor (mAChR) subtypes may interact with caveolae still warrants investigation. Using both molecular and functional approaches, this study investigates the potential link between caveolae and contractile responses induced by muscarinic activation in the bladder.

Study design, materials and methods

The molecular interaction between M_3 and M_2 mAChR subtypes and caveolin-1 (Cav-1, the principal scaffolding protein in caveolae) was examined by co-immunoprecipitation of lysate from rat bladder tissue without mucosa. For functional studies, longitudinal rat bladder tissue strips were equilibrated under 1.5 grams of resting tension in organ baths containing Kreb's solution at 37ºC. mAChR activation was achieved by stimulating tissue with cumulative concentrations of carbachol (CCh, 1nM-10µM). Dose response curves were generated under baseline conditions as well as in the presence of mAChR antagonists 4-DAMP (10nM) or AFDX (0.1µM) to partially inactive mAChRs and thus promote predominantly M_2 or M_3 mediated responses, respectively. Subsequently, bladder tissue was incubated with methyl-β-cyclodextrin (mβCD, 15mM 1½ hour), an agent that disrupts caveolae integrity by depleting membrane cholesterol, and then responses to CCh were repeated.

Results

Immuno-reactive bands corresponding to M_3 and M_2 mAChR subtypes (75 kDa and 55 kDa respectively) were detected in Cav-1 immunoprecipitates prepared from bladder tissue. The contractile response to CCh (in the absence of antagonists) was not affected by mβCD treatment. Compared to baseline responses, 4-DAMP decreased the amplitude of CCh-induced contractions at each dose. After caveolar depletion by mβCD and in the presence of 4-DAMP, the contractile response to CCh was significantly enhanced. The administration of AFDX had little effect on the dose response curve to CCh compared to baseline responses. Subsequent caveolar depletion achieved by mβCD in the presence of AFDX attenuated the amplitude of contractions induced by CCh.

Interpretation of results

The co-precipitation of M_3 and M_3 mAChRs with Cav-1 indicates the molecular interaction between these proteins and suggests the localization of these receptors in caveolae membrane domains. The opposite impact of mβCD on CCh responses in the presence of M_2 or M_3 antagonists suggests that caveolae negatively regulate M_2 mediated signaling and positively regulate M_3 mAChR mediated signalling. This complex interaction is masked when only the aggregate effect of CCh is examined.

Concluding message

The molecular and functional results from this study are consistent with the hypothesis that caveolae differentially modulate detrusor responses induced by muscarinic receptor subtype activation. Thus changes in the balance among caveolin-mAChR interactions, due to loss of caveolae or changes in mAChR subtype expression may significantly alter the functional response to cholinergic neurotransmission.

References

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Disclosures

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