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Wuest M[1], Eichhorn B[1], Kaumann A[2], Ravens U[1]
1. Department of Pharmacology and Toxicology, Medical Faculty, Dresden University of Technology, 2. Department of Physiology, Development and Neuroscience, University of Cambridge

MURINE DETRUSOR RELAXATION IS MEDIATED THROUGH (2–ADRENO-CEPTORS AND UNRELATED TO CAVEOLAE

Hypothesis / aims of study

Urinary bladder smooth muscle relaxation can be mediated via β-adrenoceptors (β-AR).[1] Function of β_1- and β_2-AR was reported to be associated with caveolae in the cardiovascular system.[2] Caveolae are flask-shaped plasmalemmal invaginations which are stabilized by members of the caveolin protein family. Genetic deletion of the caveolin-1 (cav-1) protein results in a loss of the caveolae structures in smooth muscle cells.[3] In this study we have investigated the role of β-AR subtypes mediating detrusor relaxation in wild type (WT) and cav-1 knockout (cav-1 KO) mice.

Study design, materials and methods

Relaxant responses to β-AR stimulation with (-)-isoprenaline were measured in urothelium-denuded detrusor strips precontracted with 40 mM KCl. Experiments were carried out in the presence of phentolamine, 3 μM, to block α-adrenoceptors. Four strips were usually prepared from the detrusor of one mouse. Long lasting 40 mM KCl contractures were produced on all strips: one served as a time-matched control, another for a control concentration-response curve (CRC) for (-)-isoprenaline, and the remaining two for CRC of (-)-isoprenaline in the presence of antagonists. The following antagonists, pre-incubated for 1h, were used as tools to assess the role of β-AR subtypes: β_1-AR-selective CGP 20712A (CGP; 300 nM), β_2-AR-selective ICI 118,551 (ICI; 50 nM) and β_3-AR-selective L-748,337 (L; 100 nM). Experiments were terminated by inducing nearly complete relaxation of KCl contractures in both WT and cav-1 KO with the adenylyl cyclase activator forskolin (10 μM). Total mRNA expression of β-ARs was determined in WT and cav-1 KO mice detrusor using quantitative RT Real-time PCR.

Results

Contractile dysfunction was noted in detrusor strips from cav-1 KO mice. KCl-evoked contractions were reduced after 45 min to 0.51 ± 0.05 (n = 92/27) vs 0.98 ±0.06 (n = 139/43) $mN \cdot mg^{-1}$ wet weight (ww) in WT ($p < 0.05$). Forskolin relaxed contractions to the same absolute value: 0.11 ± 0.03 vs 0.10 ± 0.02 $mN \cdot mg^{-1}$ ww in cav-1 KO and WT respectively.

(-)-Isoprenaline relaxed detrusor precontracted with with 40 mM KCl with a potency of $-\log EC_{50}$ [M] 8.04 ± 0.08 (n = 34) in WT and 7.76 ± 0.15 (n = 19) in cav-1 KO mice ($p > 0.05$). The selective β_1-AR blocker CGP 20712 did not significantly affect the CRC for (-)-isoprenaline in strips from WT and cav-1 KO. In contrast, the selective β_2-AR blocker ICI 118,551 caused shifts of the CRC for (-)-isoprenaline to higher concentrations in strips from WT ($pK_B = 9.32 \pm 0.24$) and cav-1 KO ($pK_B = 9.58 \pm 0.25$). The β_3-AR blocker L 748,337 did not affect the CRC of (-)-isoprenaline for neither WT nor cav-1 KO.

The selective β_3-AR agonists BRL 37,344 and L 755,507 both induced detrusor relaxation in WT and cav-1 KO mice, but their effects were not affected by β-AR antagonists selective for β_1-AR, β_2-AR and β_3-AR. Under preferential β_2-AR conditions (in the presence of the β_1-AR antagonist CGP 20712 and β_3-AR antagonist L 748,337) the β_2-AR antagonist ICI 118,551 also shifted the CRC for (-)-epinephrine to higher concentrations resulting in a $pK_B = 9.58 \pm 0.07$ in WT mice.

In addition we have also determined the total mRNA expression of the three β-AR in both WT and cav-1 KO detrusor tissue. β_1-AR mRNA was the most dominant subtype expressed in WT, while mRNA levels of β_2 and β_3 were about 6 to 10-fold lower. Interestingly, expression of mRNA for β_1-AR and β_2-AR was unchanged in cav-1 KO, whereas β_3-AR mRNA was significantly ($p < 0.05$) reduced in the cav-1 KO tissue.

Interpretation of results

Detrusor relaxation in the mouse urinary bladder is almost exclusively mediated via β_2-ARs. No functional involvement of either β_1-ARs or β_3-ARs was detectable. The genetic deletion of cav-1 in the cav-1 KO mice resulted in a contractile dysfunction as shown after stimulation with KCl, which mainly activates postsynaptic voltage-dependent Ca^{2+}-channels. β-AR mediated detrusor relaxation induced by different β-AR agonists was always less effective in cav-1 KO mice compared to WT indicating an overall dysfunction in relaxation as well. In contrast, the β-AR induced relaxation is mostly mediated by β_2-ARs in both cav-1 KO and WT, which is seen in the similar affinity values for ICI 118,551. The observed significant lower expression of β_3-ARs in cav-1 KO seems to have no functional impairment on the β-AR mediated detrusor relaxation in the mouse.

Concluding message

We conclude that catecholamine-evoked detrusor relaxation in the mouse is mostly mediated through β_2-AR in both WT and cav-1 KO mice. β_2-AR function is unperturbed by loss of caveolae.

References

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