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CONTRACTILE ROLE OF M2 MUSCARINIC RECEPTORS IN URINARY BLADDER SMOOTH MUSCLE

Hypothesis / aims of study

The aims of our study are to investigate the contractile role of M_2 muscarinic receptors in mouse urinary bladder. Our hypothesis is that M_2 receptors in the urinary bladder mediate an inhibition of the relaxant effects of isoproterenol and forskolin on contractions elicited by other agents.

Study design, materials and methods

Contractility measurements were made on isolated urinary bladders from male, wild type C57BL/6 mice and on male M_2 muscarinic receptor knockout (M_2 KO), M_3 KO and M_2/M_3 double KO mice. Mice were euthanized, and urinary bladders were removed and mounted in organ baths containing Krebs Ringer Bicarbonate (KRB) buffer, indomethacin (1 μM) and tetrodotoxin (0.1 μM) as described previously [1]. The contractile response to a fixed concentration of $PGF_{2\text{-alpha}}$ was measured in urinary bladders from M_3 KO and M_2/M_3 KO mice. After a steady contraction was attained, isoproterenol (1 μM) was added. After complete relaxation was attained, a cumulative concentration-response curve to the muscarinic agonist, oxotremorine-M was measured with $PGF_{2\text{-alpha}}$ and isoproterenol still present in the bath. The experiment was repeated using forskolin (10 μM) as the relaxant agent. In experiments on wild type and M_2 KO mice, urinary bladders were first incubated with 4-DAMP mustard (10 nM) in combination with the M_2 selective antagonist, AF-DX 116 (1 μM), for a total of two hr to inactivate M_3 muscarinic receptors selectively [2]. The urinary bladders were washed extensively, and the contractile responses to oxotremorine-M were measured under the conditions described above except that KCl (37.5 mM) was used instead of $PGF_{2\text{–alpha}}$. The agonist concentration-response curves were analyzed by nonlinear regression analysis to determine the maximal contractile response (E_{max}) and the negative logarithm of the concentration of agonist eliciting a half-maximal response (pEC_{50}).

Results

$PGF_{2\text{-alpha}}$ exhibited little or no contractile activity in urinary bladders from wild type and M_2 KO mice. In urinary bladders from both M_3 KO and M_2/M_3 KO mice, $PGF_{2\text{-alpha}}$ elicited a robust contractile response characterized by E_{max} and pEC_{50} values of 6.7 g and 6.38 in M_3 KO mice, respectively, and 7.2 g and 6.60 in M_2/M_3 double KO mice.

When measured in urinary bladder from M_3 KO mice in the presence of $PGF_{2\text{-alpha}}$ (1 μM) and isoproterenol (1 μM), oxotremorine–M elicited a potent contractile response characterized by a pEC_{50} value of 6.86 and an E_{max} value of 114%, expressed relative to the contraction elicited by KCl (50 mM). When similar experiments were run on M_3 KO mice using forskolin (10 μM) as the relaxant agent, the pEC_{50} and E_{max} values of oxotremorine–M were 6.60 and 98%, respectively. In contrast, when either isoproterenol or forskolin was used as the relaxant agent under the same conditions in urinary bladder from M_2/M_3 double KO mice, oxotremorine-M was without effect.

When measured in urinary bladder from wild type mice after 4-DAMP mustard-treatment and in the presence of KCl (37.5 mM) and isoproterenol (1 μM), oxotremorine-M elicited a contractile response characterized by pEC_{50} and E_{max} values of 6.01 ± 0.15 and 302 ± 36%. The corresponding values in M_2 KO mice were significantly ($p < 0.05$) smaller: 5.63 ± 0.14 and 151 ± 29%, respectively. When these experiments were repeated using forskolin (10 μM) as the relaxant agent, the pEC_{50} and E_{max} values of oxotremorine-M in urinary bladder from wild type mice were 5.90 ± 0.13 and 284 ± 5.3, respectively. The corresponding values in M_2 KO mice were 5.69 ± 0.11 and 88 ± 14%, respectively. The reduction in E_{max} in the M_2 KO mouse was highly significant ($p = 0.006$).

Interpretation of results

Our results show that the sensitivity of the mouse urinary bladder to $PGF_{2\text{-alpha}}$ greatly increases in male M_3 KO and M_2/M_3 mice. This increase in sensitivity to $PGF_{2\text{-alpha}}$ may be

triggered by the distention in the urinary bladder that occurs in male mice lacking M_3 receptors [3].

Although muscarinic agonists elicit little contractile response in urinary bladder from M_3 KO mice [3], oxotremorine-M elicits a robust contractile response in this tissue in the presence of isoproterenol and $PGF_{2\text{-alpha}}$. When present in combination, $PGF_{2\text{-alpha}}$ and isoproterenol have no net contractile effect because the contractile action of $PGF_{2\text{-alpha}}$ is offset by isoproterenol. This contraction is mediated by the M_2 receptor because this response is completely lost in M_2/M_3 double KO mice. Consequently, our results suggest that the M_2 receptor can mediate contraction by inhibiting the relaxant effect of isoproterenol on $PGF_{2\text{-alpha}}$-induced contractions. A similar conclusion can be drawn with regard to the relaxant effects of forskolin. Our results also show that activation of M_2 receptors can inhibit the relaxant effects of isoproterenol and forskolin in urinary bladder from wild type mice.

Concluding message

Since the M_2 receptor has a contractile role in urinary bladder smooth muscle, it is conceivable that antagonists with selectivity for both M_2 and M_3 muscarinic receptors may be useful in the treatment of urinary incontinence.

References

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