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LARGE SPECIES DIFFERENCES IN CONTRIBUTION OF CALCIUM-INFLUX VIA L-TYPE CALCIUM CURRENTS DURING MUSCARINIC RECEPTOR MEDIATED DETRUSOR CONTRACTION

Hypothesis / aims of study

Carbachol (CCh)-induced detrusor contractions are mainly mediated via M_3 receptor subtype[1] and depend not only on Ca^{2+}-release from the intracellular Ca^{2+} stores but also on Ca^{2+}-influx via L-type Ca^{2+}-channels. The proposed cellular mechanism for M_3-mediated contractions involves inositol trisphosphate (IP_3)-induced Ca^{2+} release from intracellular stores, Ca^{2+}-influx via nifedipine-sensitive L-type Ca^{2+}-channels and increased sensitivity of the contractile machinery to Ca^{2+} via inhibition of myosin light chain phosphatase through activation of rho kinase.[2] The contribution of Ca^{2+}-influx was recently evaluated in Ca^{2+}-channel ($Ca_v1.2$) knockout mice which had significantly smaller contractions induced by CCh compared to wildtype.[3]
The purpose of this study was to examine the different contribution of Ca^{2+}-influx and possible other mechanisms underlying muscarinic receptor-mediated contractions in human, pig and mouse detrusor.

Study design, materials and methods

Contractility was measured in urothelium-denuded muscle strips from patients undergoing radical cystectomy for treatment of urinary bladder cancer; from pigs and from C57Bl6 mice.
Contractions were induced by increasing concentrations of the muscarinic receptor agonist CCh. After equilibration, cumulative concentration response curves (CRC) for CCh were generated followed by a washout period of 1 hour. Then test drugs were added and a second CRC for CCh in the presence of the test compound was started after one further hour. Time-matched control (TMC) experiments were run separately without any drug added.

Results

The L-type Ca^{2+}-channel blocker nifedipine impaired detrusor contraction in man by no more than 25 % but by 80 to 90 % in pig and mouse resulting in the following order of efficacy in the three studied species: man < pig = mouse. The effects on maximum responses were comparable in pig and mouse detrusor. On the other hand, contractions elicited by high KCl were blocked to 75-80 % by nifedipine in all three species, confirming their Ca^{2+}-influx-dependent nature.
2-aminoethoxydiphenyl borate (2-APB) impaired CCh-induced contractions to various degrees: 300 µM 2-APB blocked 40% of maximum response in man, 65% in pig, but about 80% in mouse. In addition, 300 µM 2-APB tended to shift the CRC for CCh to higher concentrations in human and pig detrusor, although it did not reach the level of statistical significance. 300 µM 2-APB also completely reduced nifedipine-insensitive contractions in human detrusor. Block of the Ca^{2+}-induced Ca^{2+}-release with ryanodine had no significant effect on CCh contractions in all three species.
In pig about 50% of CCh-induced contraction amplitude was suppressed by thapsigargin (0.3-10 µM). Thapsigargin impaired CCh-induced force of contraction to a lesser extent in human and pig than in mouse detrusor, in which addition of CCh in the presence of 1 or 10 µM thapsigargin did not further increase contraction. 10 µM thapsigargin did not further reduce nifedipine-insensitive contractions in human detrusor.

Interpretation of results

In the present study, block of Ca^{2+}-influx indeed suppressed CCh-induced contractions, and did so to a lesser degree in human than in pig and mouse detrusor with complete block of contraction by nifedipine in the latter species. On the other hand, contractions elicited by high KCl were blocked similarly by nifedipine in all three species, confirming their Ca^{2+}-influx-dependent nature. This suggests that muscarinic receptor-stimulated contractions of human detrusor are less dependent on Ca^{2+}-influx via L-type Ca^{2+}-channels than in pig and mouse.
Beside inhibition of IP_3-induced Ca^{2+}-release 2-APB is also known to influence $[Ca^{2+}]_i$ by (i) direct inhibition of store-operated Ca^{2+}-influx or (ii) activation of a novel Ca^{2+}-permeable cation channel. Since influence of store-operated channels and phospholipase C on muscarinic receptor-mediated human detrusor contraction was excluded,[2] the other two mechanisms could be involved. Due to the fact that nifedipine has only little effect in human detrusor and 2-APB reduces nifedipine-insensitive contractions, we conclude, that beside Ca^{2+}-influx via L-type Ca^{2+}-channels additional processes are involved for elevating the intracellular Ca^{2+}-concentration during muscarinic receptor mediated detrusor contraction in this species.
Lack of sensitivity of contraction to ryanodine suggests, that Ca^{2+}-induced Ca^{2+}-release probably does not contribute to CCh-mediated detrusor contractions in the three species.
Our measurements with thapsigargin reveal that at the used concentrations it may directly inhibits Ca^{2+}-influx via L-type calcium currents. Other reports have predicted that thapsigargin may also stimulates release of arachidonic acid.

Concluding message

In conclusion, muscarinic receptor-mediated contractions in human, pig and mouse detrusor depend on Ca^{2+}-influx via L-type Ca^{2+}-channels but to a different extent. Beside the possible involvement of IP_3-induced Ca^{2+}-release it cannot be excluded that additional processes may play a role for elevating the intracellular Ca^{2+}-concentration during muscarinic receptor mediated detrusor contraction. Our study has shown considerable species differences between man, pig and mouse and it may has relevance for drawing conclusions about human urinary bladder contraction when using different animal models.

References
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