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ORGAN AND SPECIES DIFFERENCES IN MUSCARINIC RECEPTOR MEDIATED SMOOTH MUSCLE CONTRACTILE SIGNAL TRANSDUCTION.

Hypothesis / aims of study

An underlying hypothesis in the use of animal models of human diseases, is that mechanisms uncovered using the animal model are applicable to human. An aim of this study is to test this hypothesis as it applies to the mechanisms of contractile signal transduction mediated by muscarinic receptors in the urinary bladder. An additional aim is to determine whether there are differences in these mechanisms in different smooth muscle organs in a given species. This was accomplished using bladder and stomach smooth muscle from M_2 knockout, M_3 knockout and wild type mice and results were compared with our previous data from rat and human bladder smooth muscle. Antimuscarinic drugs are currently the mainstay of therapy for the overactive bladder (OAB) because stimulation of muscarinic receptors stimulates human bladder contraction. The previous assumptions underlying this are that abnormal bladder contractions are responsible for the symptoms of OAB and the mechanism of contraction occurs by overactivity of the normal contractile mechanism. An additional aim of these studies was to explore the validity of these assumptions.

Smooth muscle strips were suspended under 1 gram basal tension and after a 30 minute equilibration period were stimulated by exposure to isotonic Tyrode's solution containing 120 mM KCl. After peak tension was recorded, the strips were washed 3 times with normal Tyrodes and then separate groups of 4-10 strips were exposed to one of the enzyme inhibitors (tables 1 and 2) with and without 10 nM darifenacin (M_3 selective muscarinic antagonist). These concentrations of enzyme inhibitors were used for maximal effect on the target enzymes and selectivity over the others. After 30 minutes, a cumulative concentration response curve (CRC) to carbachol was determined and results were expressed as a percentage of the initial KCl response.

Results

The effect on the maximal carbachol induced contraction due to inhibition of various enzymes thought to be involved in the contractile signal transduction cascade are summarized in figures 1 (bladder) and 2 (stomach). In the bladder, inhibition of ROCK with Y-27632, PC-PLC with D609 and ROCK, PKA and PKG with H89 reduced the maximal contraction in the wild type strains as well as the M_2 KO strain. Inhibition of these enzymes completely blocked the meager cholinergic contraction in the M_3 KO strain. In the stomach body, inhibition of ROCK with Y-27632, PC-PLC with D609 and ROCK, PKA and PKG with H89 reduced the maximal contraction in the wild type strains. A similar trend, which did not reach statistical significance, was seen in the M_3 KO strain. In the M_2 KO strain, inhibition of PKC with chelerythrine, PC-PLC with D609, and ROCK, PKA and PKG with H89 decreased the maximal contraction but inhibition of ROCK selectively with Y-27632 did not decrease maximal contraction.

Study design, materials and methods

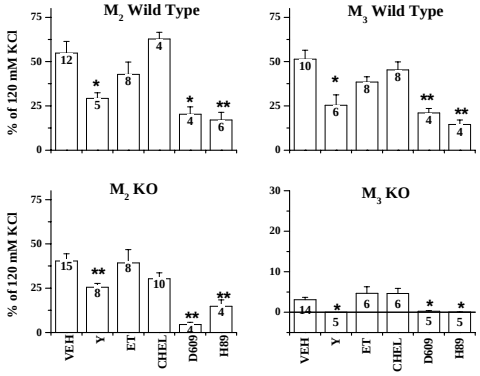
Table 1. K_i (concentration used) for Lipase Inhibitors (μM)

Inhibitor	PI-PLC	PC-PLC
ET-18-OCH_3 (ET) (100)	5	
D609 (100)		94

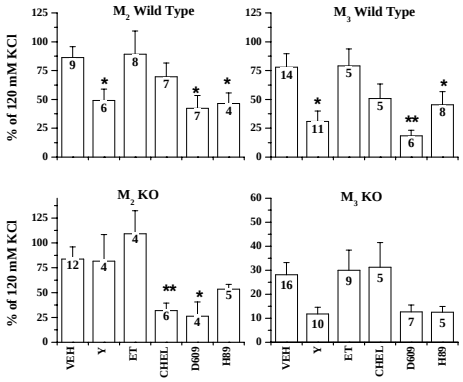
Table 2. K_i (concentration used) for Kinase Inhibitors (μM)

Inhibitor	PKA	PKG	PKC	ROCK
Y-27632 (10)	25		26	0.1
CHEL (10)			0.66	
H89 (10)	0.05	0.5		0.27

Bladder



Stomach



Interpretation of results

Results in the bladder suggest that although both the M_2 and M_3 receptor subtypes induce rho kinase and PC-PLC activation, the M_2 receptor subtype seems completely dependent upon rho kinase activation to induce contraction. This is similar to our results in human bladder but different than the normal rat bladder in which Y-27632 has no effect on maximal carbachol induced contractions. The M_3 receptor subtype likely utilizes additional signal transduction mechanism to mediate contraction. Because the M_2 mediated contractions in the M_3 KO mouse bladder were completely inhibited by the inhibitors that did not completely block the M_3 mediated contractions in the M_2 KO strains,

although both the M_2 and the M_3 receptor subtypes mediate similar signal transduction cascades in the bladder, the M_2 subtype appears less efficient at activating these cascades than the M_3 receptor subtype.

Muscarinic receptor mediated contractile signal transduction mechanisms are different in the stomach. In the M_2 KO strain, inhibition of PKC with chelerythrine reduces maximal contraction, suggesting that the M_3 receptor subtype activates PKC in the stomach but not in the bladder. Additionally, inhibition of rho kinase with Y-27632 has no effect on stomach contractions in the M_2 KO strain (but does inhibit bladder contractions) suggesting that either the stomach M_3 receptor subtype does not utilize rho kinase or other redundant signal transduction mechanisms are activated which mediate contraction. The differences in effect of the enzyme inhibitors between the KO strains and the wild type strains in which both receptor subtypes are activated suggest a complex interaction between signal transduction mechanisms activated by muscarinic receptor subtypes.

Concluding message

These results indicate that not only is the muscarinic receptor mediated contractile signal transduction mechanism different in the same organ such as the bladder across different species (mouse, rat and human) but the signal transduction in the same species (mouse) is different across different smooth muscle organs (bladder versus stomach). In addition previous studies have shown that the signal transduction mechanisms in the same smooth muscle organ (bladder) in a given species (rat) is different under different experimental conditions (normal versus hypertrophied). Therefore the assumption that the contractile signal transduction mechanism of the motor overactive human bladder occurs through the same signal transduction mechanism as normal human bladder contraction is almost certainty false.

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