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WHICH MUSCARINIC RECEPTOR SUBTYPE MEDIATES CONTRACTION OF THE HUMAN URINARY BLADDER?

Hypothesis / aims of study

Although it is generally accepted that cholinergic contraction of the normal human urinary bladder is mediated by the M_3 muscarinic receptor subtype, this conclusion is actually based on very few reports in a very limited total number of human bladder specimens (1-3). The aim of this study is to determine the muscarinic receptor subtype mediating human bladder contraction based on the affinity of M_3 subtype selective antagonists (darifenacin or p-F-HHSiD) for inhibition of cholinergic contractions in a large number of human bladder specimens obtained from brain dead organ transplant donors.

Study design, materials and methods

Whole urinary bladders were obtained from organ transplant donors and transported to the laboratory by overnight courier at 0C in ViaSpan® (Belzer UW) transport media. Bladder smooth muscle strips from 36 different donors 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 either the M_3 selective muscarinic antagonists darifenacin (n=30) or p-F-HHSiD (n=6) or vehicle. After 30 minutes, a cumulative concentration response curve (CRC) to carbachol was determined and the affinity of the antagonist was determined. For further analysis, the bladders were separated according to M_3-selective antagonist affinity as either low affinity (consistent with M_2 receptors mediating contraction), intermediate affinity or high affinity (consistent with M_3 receptors mediating contraction). The outcome measures for the bladders is the affinity of darifenacin for inhibiting contraction, the maximal carbachol mediated contraction and the potency of carbachol for mediating contraction. Muscarinic receptor subtypes were quantified in both muscle and mucosa from 11 of these bladder specimens by subtype selective immunoprecipitation.

Results

As can be seen in figure 1, the darifenacin affinity is low in 1/3, intermediate in 1/3 and high in 1/3 of the specimens. There is no difference in age, race, or sex between groups. There is no difference in maximal contraction between the groups, however, carbachol potency is significantly reduced in the intermediate and low affinity bladders compared to high affinity bladders. As a consequence there is a significant correlation ($r=0.5$, $p<0.01$) between carbachol potency and darifenacin affinity (figure 2). As can be seen in figure 3, there is no difference in detrusor muscle muscarinic receptor subtype density between groups, however, there is a significant upregulation of total, M_2 and M_3 receptors in the mucosa from the low and intermediate affinity bladders.

Figure 1

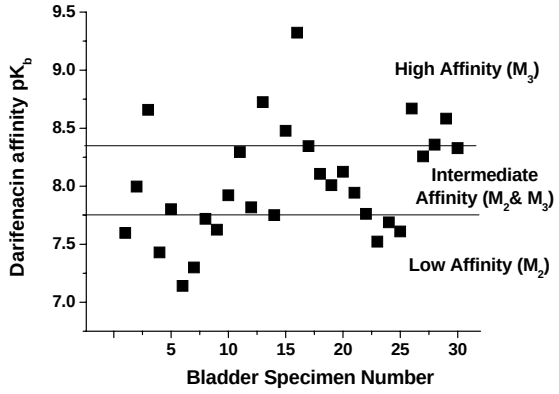


Figure 2. Human Bladders - Correlation of Darifenacin Affinity with Carbachol Potency

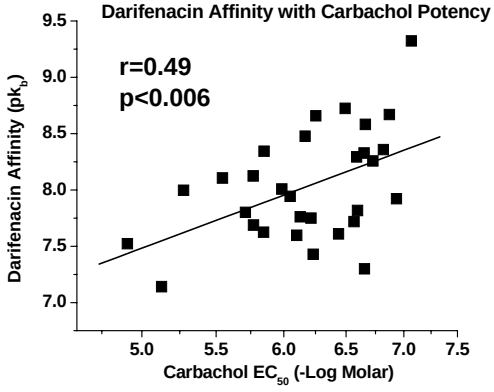
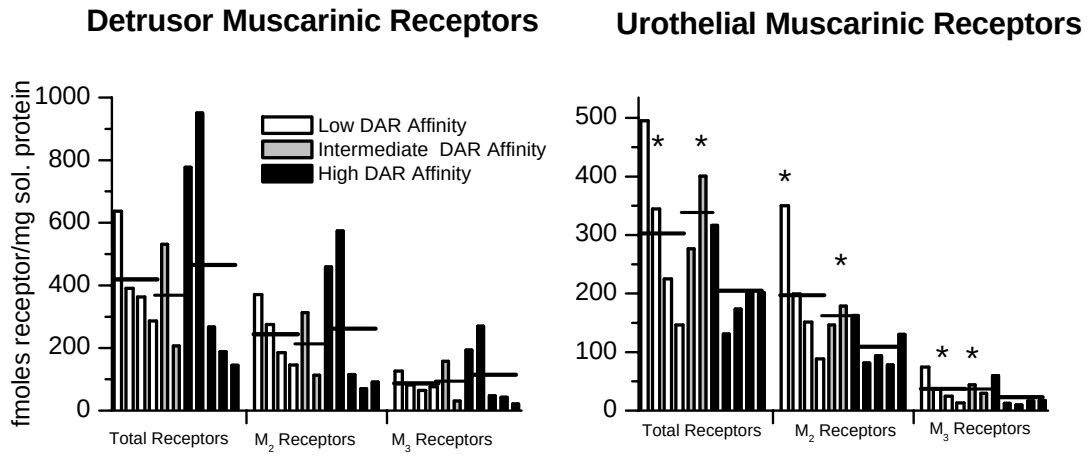


Figure 3.



Interpretation of results

These findings in this relative large series of relatively normal human bladder specimens obtained from organ transplant donors is in contrast to previous reports which found that the M_3 receptor mediates cholinergic contractions in normal human bladder. These previous reports have pooled data from different patient specimens, reporting average affinity for the subtype selective antagonists across the different donor specimens with the underlying assumption that the muscarinic receptor subtype mediating contraction is the same among the different human bladder specimens. Our results indicate that relatively normal bladder specimens obtained from brain-dead organ transplant donors can be mediated by M_2 receptors, M_3 receptors or a combination of M_2 and M_3 receptors. The bladders with low contractile affinity for M_3 selective antagonists are associated with a decreased potency of carbachol and an up regulation of the density of total M_2 and M_3 receptor subtypes in the mucosa but not the muscle.

Concluding message

In 36 bladder specimens from organ transplant donors the M_3 receptor subtype mediates contraction in 1/3 of the specimens, the M_2 receptor mediates contraction in 1/3 of specimens and a both M_2 and M_3 receptors mediate contraction in 1/3 of the specimens. The decreased carbachol potency and upregulation of mucosal muscarinic receptors in bladders with M_2 mediated contractions points towards a possible role of the urothelium in inducing a change in the phenotype of the underlying smooth muscle from contractions mediated by the M_3 receptor subtype to contractions mediated by the M_2 receptor subtype.

Literature cited:

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FUNDING: NONE
DISCLOSURES: NONE
HUMAN SUBJECTS: This study was approved by the Institutional Review Board and followed the Declaration of Helsinki Informed consent was obtained from the patients.