424

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AGE INDUCED CHANGES IN MUSCARINIC RECEPTOR SUBTYPES MEDIATING URINARY BLADDER CONTRACTION.

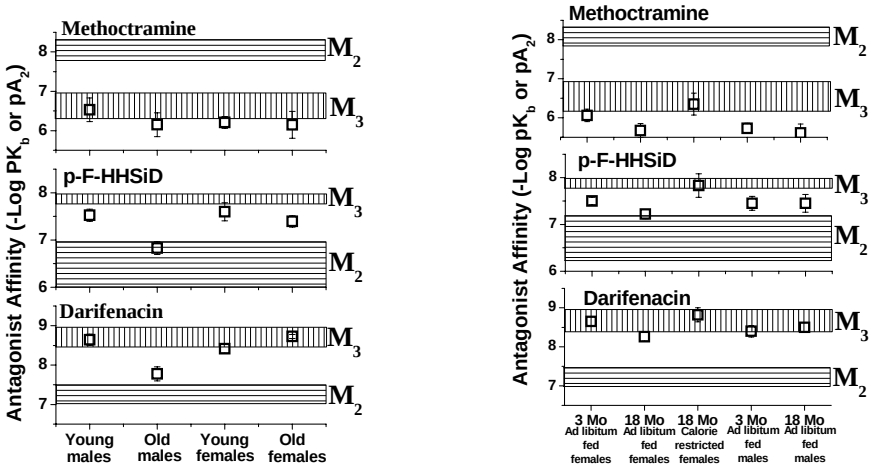
Hypothesis / aims of study

Aging is associated with a number of changes including effects on bladder function. We performed the following studies to determine whether aging in rats is associated with a change in the muscarinic receptor subtype mediating bladder contraction from M_3 towards M_2 similar to what we have previously found for bladder hypertrophy.

Study design, materials and methods

The affinities of 3 subtype selective muscarinic receptor antagonist drugs for inhibition of carbachol induced bladder contractions were determined in male and female Sprague Dawley (SD) rats at 3 months of age and greater than 12 months of age: methoctramine (M_2 selective), para fluoro hexa hydro siladifenadol (p-F-HHSiD, M_3 selective) and darifenacin (M_3 selective). This was also done on the Fisher strain of rats obtained from the United States National Institutes of Ageing (NIA) rodent colony in both 3 month and 18 month old males and females. Calorie restriction of these rats after 3 months of age increases their lifespan and the Fisher strain aged rats were obtained from the NIA colony as both ad libitum fed and calorie restricted. The SD rats were fed ad libitum. For these studies, separate groups of 6-10 bladder strips from different rats were exposed to different antagonist concentrations for 30 minutes prior to the addition of cumulative carbachol concentrations from 10 nM through 1 mM at half log intervals. Thus each muscle strip was only exposed to a single antagonist concentration and a single agonist concentration response curve (CRC). We found that exposure of muscle strips to 5 repeated carbachol CRCs modifies the contraction such that the contractile response to the 5th CRC is partially mediated by the M_2 receptor subtype.

Results



For the above graphs, the shaded areas represent the affinity ranges of subtype selective antimuscarinic agents for the M_2 and M_3 receptor subtypes reported in the literature.[1,2] There were no significant differences in the maximal contraction or the potency of carbachol in any of these groups. The left panel above shows that in the old male SD rats, the affinities of both p-F-HHSiD and darifenacin are low, consistent with M_2 mediated contractions. In both young and old female SD rats and young male SD rats, the affinities of both of these antagonists are high, consistent with M_3 mediated contractions. Since we did not find any significant differences in antagonist affinity between 3 month and 18 month old male Fisher rats fed ad libitum (right panel above), we did not perform additional studies on 18 month old male Fisher rats given a calorie restricted diet. There was a trend towards a lower affinity for all antagonists in the 18 month old ad libitum fed females Fisher rats and the affinity of p-F-HHSiD was within the range reported for M_2 receptors (right panel above).

Interpretation of results

These results show that the affinity of two different M_3 selective antagonists are consistent with M_2 mediated contractions in bladder muscle strips of aged male SD rats. This is also true for one of the M_3 selective antagonists (p-F-HHSiD) in aged female Fisher rats fed ad libitum. In all tissues tested the affinity of the M_2 selective antagonist methoctramine was low, consistent with M_3 mediated contractions. We interpret the apparent contradictory data (i.e. a low p-F-HHSiD affinity indicating M_2 mediated contractions together with a low methoctramine affinity indicating M_3 mediated contractions) to be consistent with a scenario in which either the M_2 or the M_3 receptor can mediate contraction. When the M_2 receptor is inhibited by methoctramine, the M_3 receptor mediates contraction. When the concentration of methoctramine is high enough to block both the M_2 and the M_3 receptor, contraction is inhibited. This would yield an affinity consistent with blockade of the least sensitive receptor to methoctramine, in this case the M_3. The converse is also true, i.e. p-F-HHSiD would not affect contraction until both M_2 and M_3 receptors are inhibited and thus a low affinity would be obtained.

Concluding message

The age related changes in bladder contractile function are associated with a change in the signal transduction mechanisms mediating the contraction such that, similar to bladder hypertrophy, the M_2 receptor plays a more prominent role in the contractile response.

References

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2. Caulfield MP, Birdsall NJ. International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. Pharmacological Reviews 1998;50(2):279-90.

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