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MUSCARINIC RECEPTOR SUBTYPE MEDIATING TONIC CONTRACTIONS OF THE BLADDER UROTHELIUM/SUBUROTHELIUM TO CARBACHOL

Hypothesis / aims of study

Isolated strips of bladder urothelium (urothelium and suburothelium) can produce tonic contractions following stimulation of neurokinin or muscarinic receptors [1]. These contractions persist even after removal of smooth muscle by microdisection and are probably mediated via myofibroblasts. The aim of the present study was to identify which muscarinic receptor subtype is responsible for mediating the contractions to the muscarinic receptor agonist carbachol.

Study design, materials and methods

Bladders were obtained from pigs at the local abattoir. Tissue samples were taken from the bladder dome and the detrusor muscle was removed. The strips of urothelium with underlying suburothelium were then set up in Krebs-bicarbonate solution at 37°C and gassed with 5% CO2 in oxygen. Isometric developed tension was recorded and cumulative concentration-response curves to carbachol obtained in the absence and then the presence of either the M2 receptor selective antagonist methoctramine (1-30μM) or the M3 selective antagonist 4-DAMP (3-30nM). To identify the receptor mediating contractions, the antagonist affinities were determined and compared with values for these antagonists in the literature. Responses in the absence and presence of antagonist were analysed using Students t-test.

Results

Carbachol induced contractile responses of strips of bladder urothelium and bladder smooth muscle with similar maximum responses in the two set of tissues when expressed as a percentage of the response to 75mM potassium chloride (276±38% and 204±24% respectively).

On urothelial/suburothelial strips relatively high concentrations of the M2-receptor selective antagonist methoctramine (3 & 10μM) were required to antagonize contractile responses to carbachol and the antagonist produced an affinity estimate <6.0. Increasing the concentration of methoctramine to 30μM failed to produce any further antagonism of responses to carbachol.

In contrast, the M3-receptor selective antagonist 4-DAMP caused rightward shifts of concentration-response curves to carbachol at relatively low concentrations (3-30nM) without any change in maximum response. The Schild plot was linear (slope of 0.92±0.06) and yielded a relatively high affinity estimate of 8.86±0.54. Similar affinity values were obtained when the urothelial strips were microdissected under the microscope to remove any traces of smooth muscle at the start of the experiment.

Interpretation of results

The data demonstrate that the pig bladder urothelium/suburothelium can contract in response to muscarinic receptor stimulation with carbachol. The responses appear to be mediated via the M3 receptor subtype although the lack of additional antagonism with high concentrations of methoctramine may indicate some involvement of an additional receptor subtype.

Concluding message

The study demonstrates a unique response of the bladder, namely contraction of the urothelium/suburothelium layer by a muscarinic agonist. The receptor subtype mediating these tonic contractions appears to be the M3 receptor. Since stretch of the urothelium causes release of acetylcholine [2], the results suggest that the urothelium itself may have contractile activity during bladder filling. This may offer another site of action for muscarinic receptor antagonists when used in the treatment of the overactive bladder.

References

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Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**No**
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