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M3 MUSCARINIC RECEPTOR LIKE IMMUNO-REACTIVITY (M3-IR) PATTERNS IN THE NORMAL AND PATHOLOGICAL GUINEA PIG BLADDER.

Hypothesis / aims of study

Recently it has been postulated that interstitial cells might play a role in the control mechanism of the bladder. Anticholinergic drugs presumably affect this control mechanism of the bladder to a larger extent than the detrusor muscle itself. Therefore both, the distribution of the M3 receptors in the bladder wall and the nature of any possible difference in M_3 expression to the bladder pathology resulting from obstruction have been studied here.

Study design, materials and methods

9 male guinea pigs underwent partial bladder outlet obstruction by placing a ring around the proximal urethra, while a further 7 underwent a similar procedure but without insertion of the ring (sham). 2 weeks later the animals were killed and the bladders prepared for immuno-histochemistry. Bladder sections (10 µm) were immunostained using specific antibodies to the muscarinic receptor subtype 3, to vimentin, a marker for interstitial cells, and to the non-specific neuronal marker PGP 9.5.

Results

Bladders from sham operated animals M_3-IR was associated with smooth muscle cells. In addition, M_3-IR was found on the sub-urothelial interstitial cells which lie immediately below the urothelium. The number of these sub-urothelial interstitial cells varied in different regions of the bladder wall. Vimentin positive interstitial cells were also found dispersed within the lamina propria and on the surface of the inner and outer muscle bundles. A population of these interstitial cells were M_3-IR. A population of nerve fibres (PGP 9.5 positive) in the lamina propria and in the muscle layers were M_3-IR. Punctate M_3-IR staining was detected on the ganglionic nerve cell bodies within the lamina propria, suggesting that there are fibres are releasing acetylcholine which activates the ganglion cells via M_3 receptor mediated mechanisms.

Bladders from animals with partial outflow obstruction In these bladders there was a distinct loss of nerves associated with the muscle bundles and there were few intra-mural ganglia. However, there was a striking increase in the density of vimentin positive fibres in the lamina propria and around the muscle bundles. Many of these cells were M_3-IR. The vimentin and M_3-IR positive interstitial cells in the lamina propria extend complex bifurcating processes, which appears to be continuous with vimentin positive cells running over the surface of the muscle bundles and within the muscle bundles, forming a comlex network. Vimentin positive interstitial cells can be found in groups or 'nodes' in between the muscle bundles in the outer muscle layer. These structures are associated with nerve profiles and showed M_3-IR. There are regions of the bladder wall where trabeculae were seen with a few vimentin positive structures. These were often in close apposition to trabecula with a high density of vimentin and M_3-IR positive cells. These cells send processes to the vimentin positive structures lying on the surface of the trabeculae.

Antibody specificity Pre-incubation of the M_3 antibody with the blocking peptide against which the M3 antibody was raised showed no positive immuno-reactivity. Thus, any positive staining (M_3- immuno-reactivity (M_3-IR), may be considered to represent specific antibody binding and the location of M_3 receptors.

Interpretation of results

Examination of the M3-IR reveals a complex distribution of muscarinic receptors in the bladder wall. The implication is that there are several potentially interrelated mechanisms operating involving acetylcholine and muscarinic receptors in the normal bladder. The detailed physiological functions of these different cholinergic mechanisms, especially this in the lamina propria, are largely unknown. Examination of the bladder wall of obstructed bladders demonstrates alterations to almost all of cholinergic components. The functional significance of this is not yet fully appreciated. However, the loss of cholinergic nerve fibres and ganglia accompanied by an up-regulation of cholinergic interstitial cells within the muscle layers may reflect, an alteration in the way muscle activity is generated. These changes may contribute to the functional changes underlying bladder pathology and may point to possible sites of therapeutic action of anticholinergic drugs.

Concluding message

These data demonstrate a complex distribution of muscarinic receptors in the normal bladder and significant changes in muscarinic receptor distribution in the pathological bladder. The importance of these observations for the physiology of the normal bladder and the origin and nature of bladder dysfunction cannot be overemphasised.

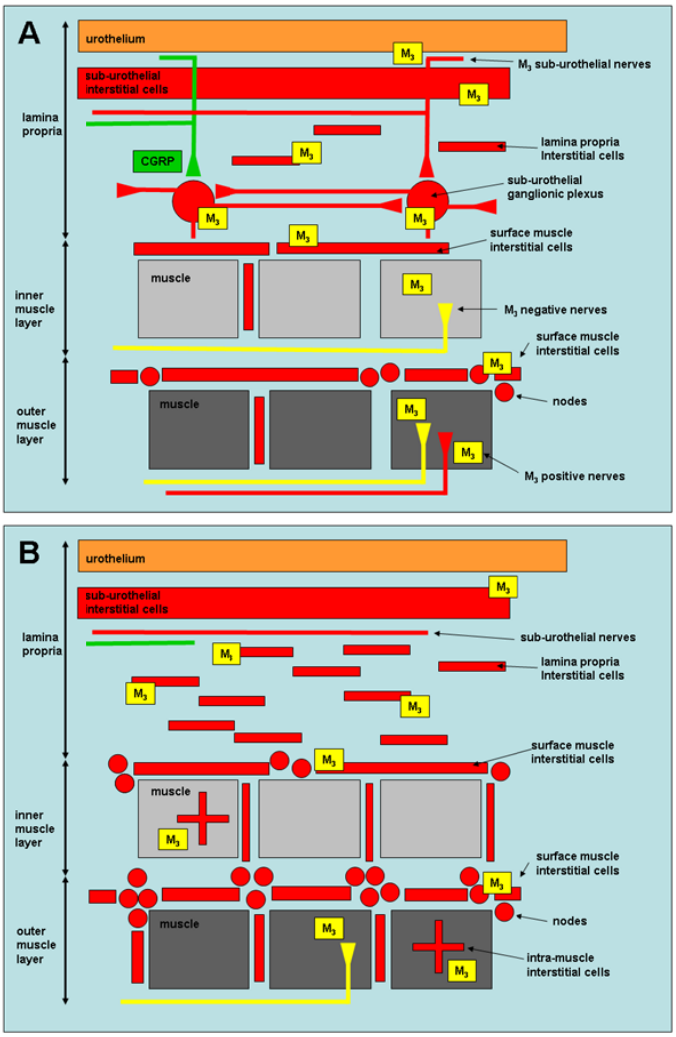

Figure 1. Schematic diagrams illustrating the observations made on the localisation of M_3-IR to cells and structures in the wall of normal bladders and bladders with previous surgical intervention to restrict the bladder neck. A shows the normal bladder. M_3-IR is located within sub-urothelial interstitial cells, cells in the lamina propria, nerves in the lamina propria, on the intra-mural ganglia, superficial muscle interstitial cells in the inner muscle layer, interstitial cells of the outer muscle layer and small nodes and to a population of nerves in the muscle bundles. B shows the situation in the obstructed bladder. Note that no ganglia are present in the obstructed bladders. M_3-IR is located in the same cell types as in the control bladders but there are differences in the number and distribution of these cells. In the OB bladders there are more M_3-IR cells dispersed within the lamina propria. M_3-IR positive superficial muscle interstitial cells are more abundant particularly in the outer region. There are also prominent nodes of M_3-IR positive cells. Muscle bundles are found which have a high incidence of intra-muscular M_3-IR positive cells while others have few such cells.

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