

## **MUSCARINIC TYPE 2 RECEPTORS ON BLADDER SENSORY NERVES : A NEW SITE OF DRUG ACTION FOR DETRUSOR OVERACTIVITY?**

### **Aims of Study**

Muscarinic receptor subtypes have been identified by different methods including radioligand binding, molecular, immunological and functional studies. In humans and pigs only the muscarinic type 2 (M2) and muscarinic type 3 (M3) appear to be present. In relative quantities the M2-receptor subtype predominates with the M2:M3 ratio of the receptors being 3:1. The role of the muscarinic receptor subtypes has been investigated using in vitro studies of porcine detrusor smooth muscle. It has been suggested that "the predominant, possibly only, muscarinic receptor subtype mediating direct contraction in the pig detrusor muscle in vitro is the M3 receptor".

A urothelium derived inhibitory factor has been described which depresses the underlying smooth muscle. However it is not clear whether this works by the M2, M3 receptors or both. If the action was M2 mediated, this could explain the inhibitory action of urothelium and could suggest an alternative mechanism of action for non specific antimuscarinic drugs.

The aim of this study is to investigate the presence of M2 receptors and whether they co-exist with sensory nerves in the urothelium and lamina propria by examining human bladder biopsy samples for anatomical evidence of muscarinic type 2 receptors (M2R) in direct relation to sensory nerves.

### **Methods**

Dome bladder biopsies were taken from women with a urodynamic diagnosis of idiopathic detrusor overactivity. Biopsies were fixed in 4% paraformaldehyde, mounted and stored at -80 °C. 50–70 µm sections were stained with the M2R rabbit antibody and stored overnight in a moist chamber at 20°C. One section on each slide was used as the negative control. Bovine anti-rabbit immunoglobulin labeled with hydrogen peroxide was added to the section and reacted with diaminobenzidine. The slides were then stained for sensory nerve endings using calcitonin gene-related peptide and stored in a moist chamber overnight at 20°C. The following day bovine anti-goat IgG-fluorescein isothiocyanate was added and the slides were mounted. Slides were read by 2 independent observers and scored using fluorescent/light microscopy. Biopsies were stained and visualized separately for sensory nerves and M2Rs. Twenty biopsies were stained for both antibodies.

### **Results**

The examination of all 20 biopsies stained for both antibodies indicated that M2R were present in the urothelium and lamina propria of the bladder but were not found in the deeper detrusor muscle layers (Fig 1). When the sections were also stained for the sensory nerves the M2 receptors were found to be in close proximity to the sensory nerves (Fig 2).

### **Conclusions**

These results demonstrate that muscarinic type 2 receptors and sensory nerves are adjacent in both the urothelium and lamina propria. This anatomical juxta-positioning suggests a possible mechanism of action for antagonists at muscarinic type 2 receptors. This interaction with sensory pathways could alter sensory thresholds and then inhibit reflex bladder activity. This would be a novel method of reducing urgency and increasing bladder capacity in patients with detrusor overactivity.

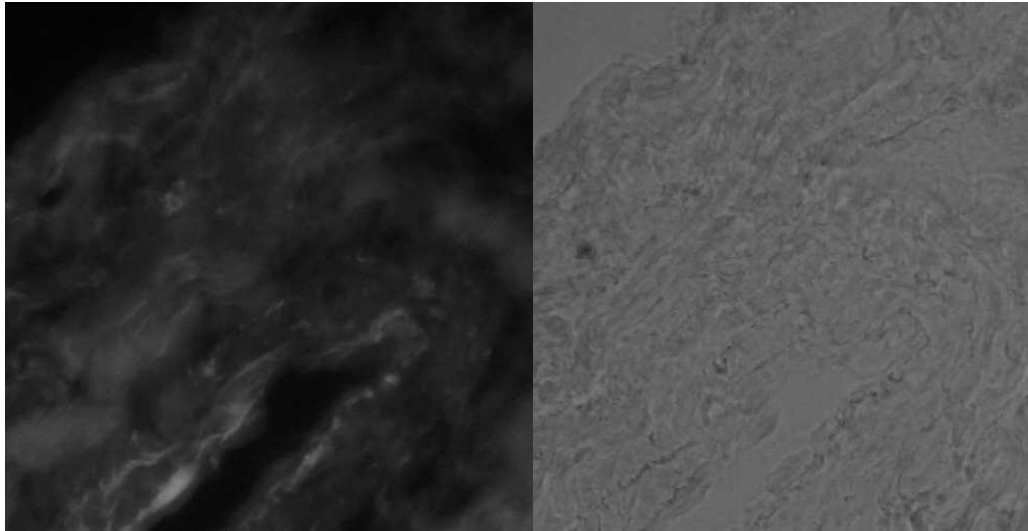


Figure 1 – CRGP stained sensory nerve

Figure 2- DAB stained M2 receptors co  
existent with sensory nerve in urothelium