

## **PRESENCE OF A NON-NEURONAL MUSCARINIC CHOLINERGIC SYSTEM IN THE STRIATED MUSCLE OF THE RAT URETHRAL SPHINCTER COMPLEX**

### **Hypothesis / aims of study**

Over the last decade evidence has accumulated for the presence of a large muscarinic cholinergic system in many non-neuronal structures (1). Recently, the co-expression of proteins that synthesize, store and bind acetylcholine in non-neuronal cells has also been demonstrated. The present study was undertaken to investigate the presence of this non-neuronal system in the individual tissue layers (urothelium, smooth and striated muscles) of the urethral sphincter complex (USC) and to quantify the distribution and expression of the different muscarinic receptor subtypes.

### **Study design, materials and methods**

Consecutive sections of USC from ten Wistar rats were immunostained and quantified by image analysis for muscarinic receptor (MR) subtypes (M1-M5), choline acetyltransferase (ChAT) and vesicular acetylcholine transporter (VAChT).

### **Results**

All cholinergic components (MRs, ChAT and VAChT) are present in the USC. Sex difference in the distribution of all components is absent. In the urothelium M<sub>1</sub>, M<sub>3</sub> and M<sub>5</sub> MRs are expressed strongly. M<sub>2</sub> and M<sub>4</sub> MRs are weakly present and absent, respectively. M<sub>4</sub> subtype is also undetectable in the muscle cells. All the other four MRs, particularly M<sub>2</sub> subtype, are expressed less intensely in the smooth muscle cells compared to the striated cells. ChAT and VAChT are expressed in the urothelium, suburothelial urethral gland and in the striated, but not smooth muscle cells of the USC (Fig. 1).

### **Interpretation of results**

The urothelium of the the USC reveals the existence of a non-neuronal muscarinic cholinergic system but its function is currently unclear.

Typically, striated muscle contraction requires release of acetylcholine from the nerve terminal to act on nicotinic receptors at the motor endplate. Recent studies have demonstrated that skeletal muscles also express muscarinic receptors (2), but their function is still uncertain. It is known that the non-innervated, peripheral part of the diaphragm and denervated striated rat muscles also express ChAT (1). Non-innervated striated muscle can thus develop a non-neuronal cholinergic system. Findings from the present study show that M<sub>1</sub>, M<sub>5</sub>, and especially M<sub>2</sub> MRs are strongly expressed in the striated muscle of the urethral sphincter complex. In addition, the striated muscle of the sphincter also contains both ChAT and VAChT, indicating that this muscle can both synthesize and store acetylcholine independent of neuronal stimulation.

The urethral sphincter complex appears, therefore, to be unique in possessing a dual mode of action which is possibly dictated by its specific function of gradually adapting the outflow resistance to increasing urine volume of the bladder through its intrinsic myogenic acetylcholine activity. During moments of increased stress additional neuronal stimulation can be called upon to prevent incontinence.

### **Concluding message**

This study demonstrates the presence of a "non-neuronal muscarinic cholinergic system" in the striated muscle of the USC. This new data could be a clue for the development of new therapeutic strategies to target the USC.

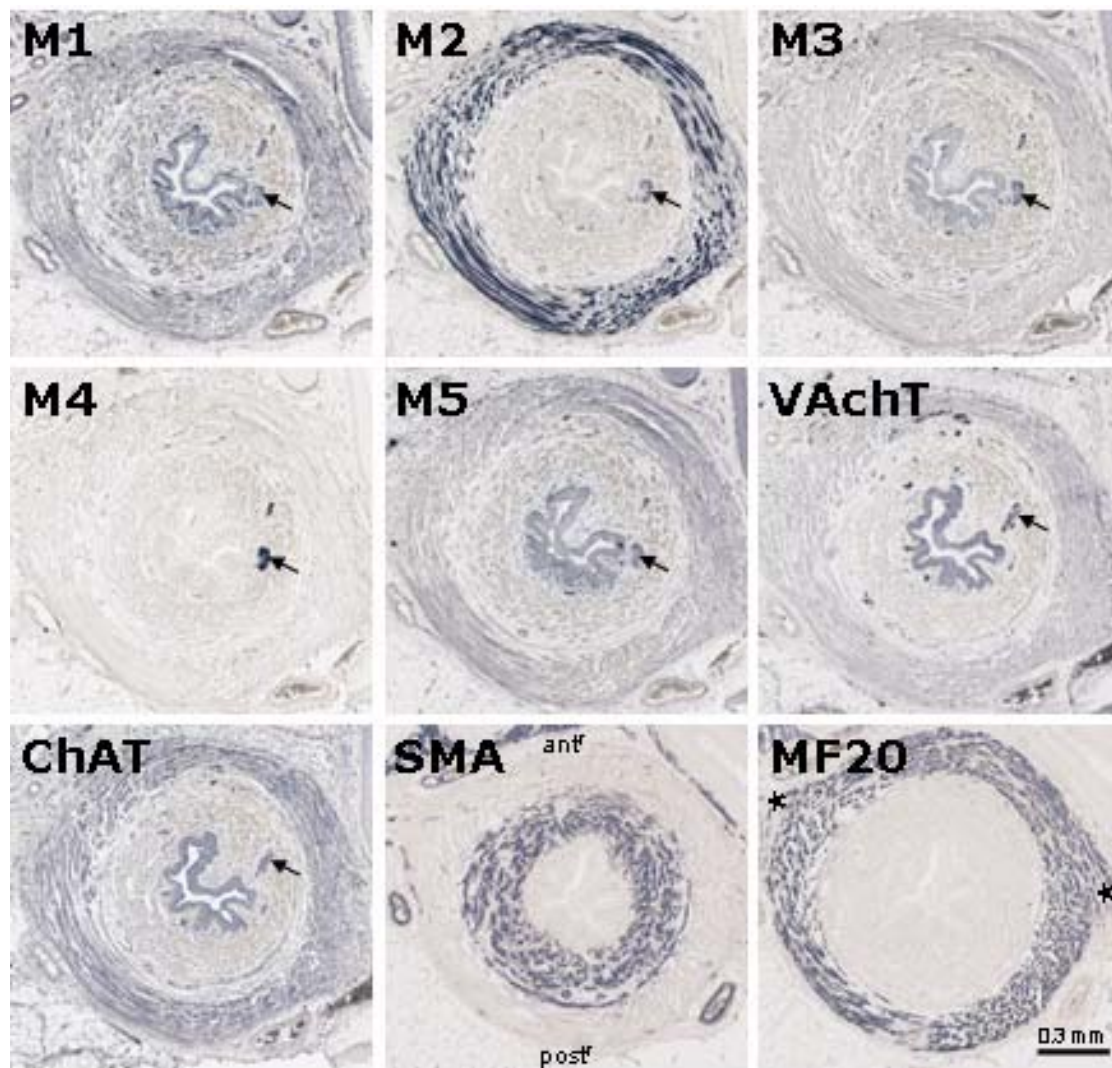


Figure 1. Expression of muscarinic receptor subtypes, choline acetyltransferase (ChAT) and vesicular acetylcholine transporter (VAChT) in the rat urethral sphincter complex. Alpha-smooth muscle actin (SMA) and myosin heavy chain (MF20) identify the presence of smooth and striated muscle fibers, respectively. Note lateral connection of external sphincter muscle with the pubocaudalis muscle (asterisks). Bar:0.3 mm.

#### References

1. The non-neuronal cholinergic system in humans: expression, function and pathophysiology. *Life Sci*, **72**: 2055, 2003
2. Functional muscarinic receptors in cultured skeletal muscle. *Arch Biochem Biophys*, **331**: 41, 1996

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