

## SPONTANEOUS CONTRACTILE ACTIVITY OF THE UROTHELIUM IS INCREASED BY MUSCARINIC AND PURINERGIC RECEPTOR STIMULATION

### Hypothesis / aims of study

Detrusor overactivity is a condition resulting from spontaneous detrusor contractions during filling but the mechanisms involved are unclear. However, the urothelium/suburothelium (urothelium) influences the detrusor and this urothelial layer is capable of spontaneously generating its own phasic contractions. The aim of this study was to identify factors that may influence the frequency of these spontaneous urothelial contractions.

### Study design, materials and methods

Isolated pig bladders obtained from the local abattoir were opened longitudinally and strips of urothelium 2cm long were taken from the anterior wall of the dome. The strips were mounted in Krebs-bicarbonate solution, maintained at 37°C and gassed with 5% CO<sub>2</sub> in oxygen. The frequencies of spontaneous contractions (cycles per minute), the amplitude of contraction (grams) and the resting basal tensions (grams) were recorded in the absence and presence of either carbachol (1µM), α,β-methylene ATP (10µM), phenylephrine (1µM), clonidine (1µM), isoprenaline (1µM) or histamine (10µM).

### Results

The urothelium exhibits spontaneous contractions within 10 minutes of being placed in the organ bath. This regular phasic activity was present throughout the course of the experiment (Figure 1).

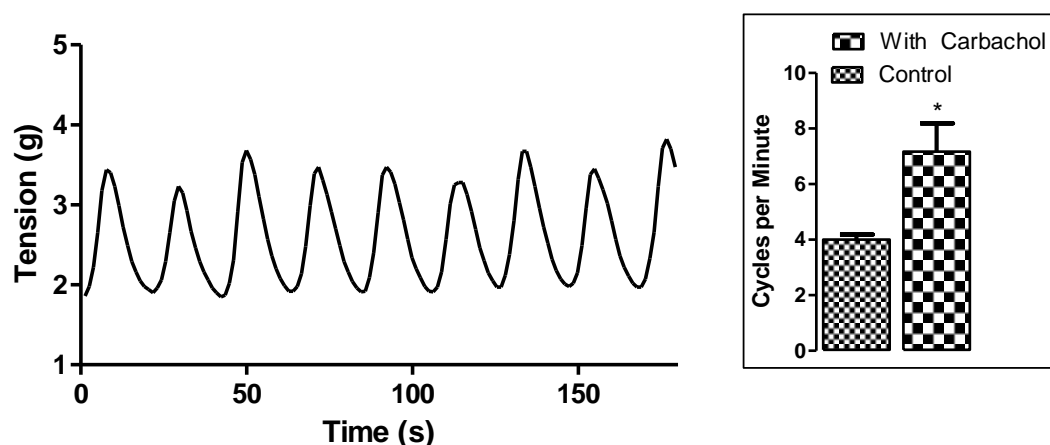


FIGURE 1: Spontaneous activity of the urothelium in the organ bath in the absence of any drug. Insert: The mean  $\pm$  SEM rate of urothelial spontaneous contractions in the absence and presence of carbachol 1µM (n = 9). \*P < 0.05 Stimulation of muscarinic or purinergic receptors with carbachol and α,β-methylene ATP respectively increased the spontaneous rate of urothelial contractions. Carbachol was found to increase the frequency by 86  $\pm$  32% (P<0.05) and α,β-methylene ATP by 33  $\pm$  7% (P<0.001). In contrast, the stimulation of adrenergic receptors (α<sub>1</sub>, α<sub>2</sub>, β) or histamine receptors did not influence the spontaneous urothelial contractile rate (Table 1). The agonists carbachol, α,β-methylene ATP and histamine also caused tonic contractions of the urothelial strips. Carbachol caused an increase in basal tension from the resting state of 149  $\pm$  18% (P<0.001), α,β-methylene ATP of 84  $\pm$  18% (P < 0.05) and histamine of 15  $\pm$  2% (P<0.001). As the urothelial strips contracted to carbachol and α,β-methylene ATP, the amplitude of spontaneous contractions was reduced; 49  $\pm$  9% (P<0.01) for α,β-methylene ATP and 28  $\pm$  9% (P<0.05) for carbachol. The other agonists had no statistically significant affect on the basal tension or the amplitude of the spontaneous contractions.

Drug	Conc	Receptors stimulated	Absence of drug	Presence of drug	n
Carbachol	1µM	Muscarinic	4.0 $\pm$ 0.3	7.2 $\pm$ 1.0*	9
α,β-methylene ATP	10µM	Purinergic	3.4 $\pm$ 0.3	4.5 $\pm$ 0.3**	8
Phenylephrine	1µM	α <sub>1</sub> -adrenergic	3.3 $\pm$ 0.3	3.6 $\pm$ 0.5	9
Clonidine	1µM	α <sub>2</sub> -adrenergic	3.5 $\pm$ 0.3	4.2 $\pm$ 0.5	9
Isoprenaline	1µM	β-adrenergic	2.9 $\pm$ 0.2	3.0 $\pm$ 0.2	8
Histamine	10µM	Histaminergic	3.5 $\pm$ 0.5	3.8 $\pm$ 0.6	12

\*P < 0.05

\*\*P < 0.001

Table 1: Mean  $\pm$  SEM spontaneous contractile rates of isolated urothelial strips (cycles per minute) in the absence and presence of each agonist.

### Interpretation of results

The urothelium exhibits phasic contractile activity and these contractions are generated spontaneously within the urothelium. Stimulation of muscarinic, and to a lesser extent, purinergic receptors, induces an increase in the frequency of spontaneous urothelial contractions. In contrast, the stimulation of adrenergic receptors or histamine receptors does not influence the frequency of urothelial phasic contractions.

### Concluding message

The demonstration of spontaneous phasic contractile activity within the urothelium identifies another function of this layer in regulating bladder activity. The results also highlight another influence that muscarinic receptors exert on the bladder and may provide a mechanism by which muscarinic antagonists act to reduce bladder overactivity.

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<b><i>Is this a clinical trial?</i></b>	<b>No</b>
<b><i>What were the subjects in the study?</i></b>	<b>ANIMAL</b>
<b><i>Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?</i></b>	<b>No</b>
<b><i>Statement that no ethical approval was needed</i></b>	<b>Ethical approval was not required as tissue samples were obtained from an abattoir (animals not killed for research).</b>