

## THE EFFECT OF RESTING TENSION, PH AND TEMPERATURE ON UROTHELIUM-DERIVED INHIBITORY FACTOR ATTENUATION OF DETRUSOR CONTRACTILITY.

### Hypothesis / aims of study

The presence of urothelium inhibits maximal bladder contractions by approximately 50% in response to carbachol in pig and human isolated muscle strips [1, 2]. This inhibitory effect has been attributed to release of a diffusible urothelium derived inhibitory factor (UDIF). The UDIF is yet to be identified but there is evidence to suggest that it is neither nitric oxide nor a cyclooxygenase product. Discovering more about the UDIF may lead to the discovery of novel targets for the clinical treatment of detrusor overactivity. The aim of this study was to identify whether the UDIF's mechanism of action or release, are altered when the general conditions of the *in vitro* experiment are changed. The effect of altering the pH, temperature and the resting tension on detrusor contractility and urothelial inhibition were observed. The results of this study may provide fundamental clues to the UDIF's identity.

### Study design, materials and methods

Paired strips of pig bladder dome with and without urothelium were set up under 1g tensions in gassed Krebs bicarbonate solution at 37°C and at pH 7.5 unless otherwise stated. In experiments investigating resting tension tissues were stretched and then kept under a resting tension of either 0.5, 1, 2.5 or 5g. The pH of the bathing solution was reduced from 7.4 to 6.9 by decreasing the concentration of the bicarbonate in the Krebs from 24mM to 6mM (values derived using the Henderson-Hasselbalch equation). In addition the physiological temperature of 37°C was compared to 27°C. Cumulative carbachol concentration response curves were obtained in the varying experimental conditions. Results were expressed as the mean  $\pm$  sem and paired Student's t-test or repeated measures ANOVA were used for statistical analysis.

### Results

**Resting tension:** In both urothelium denuded and intact strips the maximum contractions to carbachol significantly increased as the tension applied to strips is raised. However, the amount of urothelial inhibition and the detrusor sensitivity to carbachol were not significant different with varying incubation tensions.

Resting tension (g)	pEC50		Maximal contraction (g)		Urothelial inhibition (%)
	denuded	intact	denuded	intact	
0.5	5.80 $\pm$ 0.04	5.66 $\pm$ 0.03	6.7 $\pm$ 0.8	4.2 $\pm$ 0.7	38.6 $\pm$ 3.2
1.0	5.94 $\pm$ 0.06	5.70 $\pm$ 0.01	11.1 $\pm$ 2.7	5.4 $\pm$ 1.0	46.9 $\pm$ 11.0
2.5	5.80 $\pm$ 0.09	5.72 $\pm$ 0.09	25.1 $\pm$ 5.7*	10.1 $\pm$ 2.7	55.6 $\pm$ 15.9
5.0	5.89 $\pm$ 0.02	5.75 $\pm$ 0.04	29.6 $\pm$ 6.5**†	16.8 $\pm$ 4.0**†	37.5 $\pm$ 15.1

\* p<0.05 and \*\* p<0.01 indicates a significant difference from 0.5g tension. †p<0.05 indicates a significant difference from 1g tension (n=4).

**pH:** In denuded detrusor a reduction in pH from 7.5 to 6.9 caused a significant decrease in maximum contraction and pEC50 values but the urothelial inhibition was not significantly altered.

pH	pEC50		Maximal contraction (g)		Urothelial inhibition (%)
	denuded	intact	denuded	intact	
7.5	5.38 $\pm$ 0.06	5.31 $\pm$ 0.06	12.1 $\pm$ 1.5	5.3 $\pm$ 1.1	56.2 $\pm$ 6.1
6.9	5.63 $\pm$ 0.09**	5.50 $\pm$ 0.13	10.3 $\pm$ 1.2*	5.3 $\pm$ 1.0	49.4 $\pm$ 5.5

\* p<0.05 and \*\* p<0.01 indicates a significant difference from control pH 7.5. (n=6)

**Temperature:** Decreasing the temperature to 27°C significantly increased the maximal contraction to carbachol in both intact and denuded detrusor strips but the pEC50 values and the amount of urothelial inhibition was not significantly altered.

Temperature (°C)	pEC50		Maximal contraction (g)		Urothelial inhibition (%)
	denuded	intact	denuded	intact	
37	5.43 $\pm$ 0.07	5.44 $\pm$ 0.11	19.8 $\pm$ 3.5	9.0 $\pm$ 1.6	53.3 $\pm$ 6.3
27	5.57 $\pm$ 0.10	5.29 $\pm$ 0.12	32.3 $\pm$ 4.3**	14.0 $\pm$ 1.7**	47.0 $\pm$ 4.9

\*\* p<0.01 indicates a significant difference from control temperature 37°C (n=8)

### Interpretation of results

The degree of urothelial inhibition remains unaltered by changes in tension, pH and temperature, although varying these conditions has a significant effect upon detrusor contractility.

There are many reasons why the decrease in temperature could cause an increase in contractility. The major hypothesis is that enzyme activity ( $\text{Ca}^{2+}$  ATPase) would be decreased thus reducing calcium efflux from the cytoplasm. The reduction in denuded strips contractility but not in intact strips at pH 6.9 may illustrate the urothelium's barrier role in protecting the underlying detrusor and interstitial cells to changes in pH.

Altering the resting tension could cause an increase in contractility for many reasons, such as, optimum actin and myosin cross-bridging lengths, release of excitatory neurotransmitters or increased opening probability of stretch-activated cation channels.

### Concluding message

The finding that the inhibition of detrusor contraction by the urothelium remains unaltered by changes in tension, pH or temperature may provide vital clues in identifying the novel inhibitory factor. Further investigations are required to establish why a decreased temperature or increased resting tension causes an increase in detrusor contractility.

### References

1. Journal of Urology, 2003. **170** (5): p. 1897-1900.
2. British journal of Pharmacology, 2000. **129**: p. 416-419.

**FUNDING:** The Royal Hallamshire Trust

**DISCLOSURES:** NONE

**ANIMAL SUBJECTS:** This study did not follow the guidelines for care and use of laboratory animals because the tissue was obtained from an abattoir