

UROTHELIAL CONTRACTIONS TO ELECTRICAL FIELD STIMULATION ARE MEDIATED BY AN UNIDENTIFIED NEUROTRANSMITTER(S)

Hypothesis / aims of study

In recent years it has become increasingly apparent that the urothelium/suburothelium (urothelium) greatly influences both sensory and motor functions of the bladder. Also, we have recently shown that this layer exhibits contractile activity when stimulated by muscarinic or tachykinic agonists^[1]. There is evidence that a variety of different nerves innervate the urothelium and the aim of this study was to identify the neurotransmitters that cause contraction following electrical field stimulation (EFS) of the nerves in vitro.

Study design, materials and methods

Bladders were obtained from pigs at the local abattoir. Tissue samples were taken from the bladder dome and the detrusor muscle was removed. The remaining strips of urothelium were mounted in Krebs-bicarbonate solution, maintained at 37°C and gassed with 5% CO₂ in oxygen. The tissue strips were stimulated electrically to cause release of neurotransmitters and contractions were recorded. Optimal stimulation parameters were defined (20V, 0.1msec pulse width) and used to stimulate the tissues for 5 seconds every 100 seconds. Three stimulation frequencies (5, 10, 20Hz) were examined and the neurotoxin tetrodotoxin (1µM) was used to confirm the neurogenic origin of contractions.

To identify which neurotransmitters were involved in mediating contractions, atropine (1µM) was employed to antagonise muscarinic receptors, guanethadine (10µM) to block adrenergic neurotransmission, N^G-nitro-L-Arginine (L-NNA, 100µM) to block nitric oxide production and α,β-methylene ATP (10µM) to desensitise purinergic receptors. Thus, the drugs could be used to identify contributions to urothelial contractions from acetylcholine, noradrenaline, nitric oxide and ATP respectively. Data obtained in the absence and presence of drugs was analysed using paired Students t-tests.

Results

Electrical field stimulation of the urothelium resulted in contractions that were frequency dependent (Figure 1). These contractions were reproducible throughout the time course of the experiment. When the release of neurotransmitter was inhibited with tetrodotoxin (1µM), responses to electrical field stimulation were abolished. Individually, none of the drugs that affect neurotransmitter release or action influenced the amplitude of the contractions, as shown in Table 1.

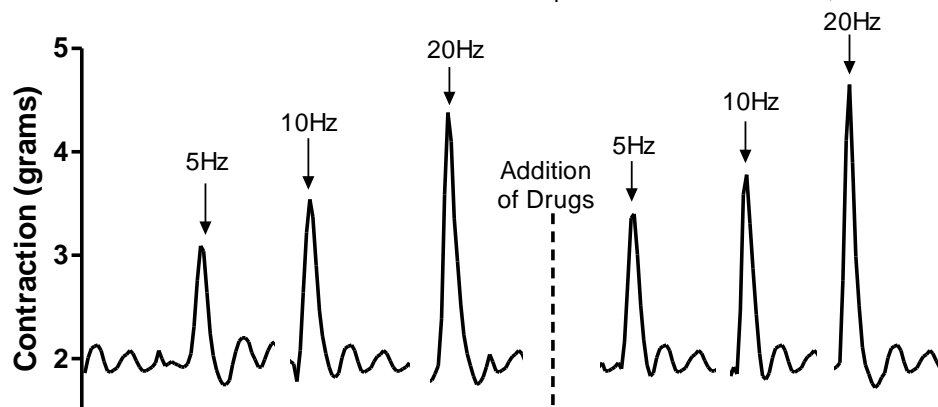


FIGURE 1: Urothelial Responses to electrical field stimulation (20 V, 1ms pulse-width) in the absence and presence of atropine (1µM), guanethadine (10µM) α,β-methylene ATP (10µM) and L-NNA (100µM).

The neurogenic contractions of the urothelium were also unaffected by a cocktail of all four blockers/inhibitors (Figure 1), responses at 5, 10 and 20Hz being $1.41 \pm 0.25g$, $2.06 \pm 0.36g$ and $2.93 \pm 0.24g$ in the absence of the drugs and $1.82 \pm 0.23g$, $2.24 \pm 0.27g$ and $2.99 \pm 0.32g$ in the presence of a combination of the drugs ($n = 13$). In contrast, these drugs significantly inhibited responses of the detrusor muscle to electrical field stimulation with identical stimulation parameters.

Drug:	n	5Hz		10Hz		20Hz	
		absence	presence	absence	presence	absence	presence
Atropine (1µM)	17	1.51 ± 0.18g	1.71 ± 0.21g	2.21 ± 0.24g	2.25 ± 0.25g	2.50 ± 0.25g	2.53 ± 0.27g
α,β-methylene ATP (10µM)	12	1.66 ± 0.25g	1.80 ± 0.28g	2.25 ± 0.32g	2.26 ± 0.29g	3.38 ± 0.47g	3.37 ± 0.40
Guanethadine (10µM)	12	1.34 ± 0.22g	1.57 ± 0.18g	1.76 ± 0.25g	1.87 ± 0.22g	2.89 ± 0.32g	3.01 ± 0.38
L-NNA (100µM)	12	1.27 ± 0.18g	1.49 ± 0.20g	1.92 ± 0.27g	2.00 ± 0.25	1.92 ± 0.32g	2.13 ± 0.28g
Tetrodotoxin (1µM)	10	1.00 ± 0.27g	0.19 ± 0.07g*	2.92 ± 0.73g	0.12 ± 0.06g**	2.30 ± 0.62	0.94 ± 0.20

TABLE 1: Mean ± SEM contractile amplitudes of urothelial strips in the presence and absence of drugs in response to electrical field stimulation at various frequencies. *P < 0.05, **P = 0.01

Interpretation of results

Electrical depolarisation of the nerves present in the bladder urothelium causes the release of neurotransmitters that result in contraction of the tissue. Unlike the detrusor muscle where acetylcholine and ATP are released as co-transmitters, the neurotransmitters in the urothelium could not be identified but do not appear to be acetylcholine, noradrenaline, ATP or nitric oxide.

Concluding message

This study demonstrates that the urothelium/suburothelium is capable of contraction and this activity can be modified by neurotransmitters released from nerves within the tissue. The neurotransmitters involved could not be identified but may represent a novel therapeutic target for the treatment of bladder overactivity.

References

1. Sadananda, P., Chess-Williams, R., Bircher, E. (2008). Contractile properties of the pig bladder mucosa in response to neurokinin A: a role for myofibroblasts? Br J Pharmacol 153(7): 1465-73

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