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Cross R L[1], Chapple C R[2], Chess-WIlliams R[3]
1. The University of Sheffield, Sheffield, UK., 2. The Royal Hallamshire, Sheffield, UK., 3. Bond University, Queensland, Australia.

IS THE UROTHELIUM-DERIVED INHIBITORY FACTOR DEPENDANT ON EXTRACELLULAR CALCIUM?

Hypothesis / aims of study

The presence of the urothelium reduces the maximal contraction of detrusor smooth muscle in response to carbachol by approximately 50% in pig and human [1, 2]. This phenomenon is attributed to the release of urothelium derived inhibitory factor (UDIF), which is currently unidentified. The UDIF may be of great clinical importance in overactive bladder (OAB) syndrome and may aid the development of novel treatments.

An influx of extracellular calcium via L-type calcium channels has been shown to account for approximately 90% of the detrusor contractile response to carbachol and potassium chloride using zero calcium solutions, knockout mice and dihydropyridine pharmacological agents [3] The aim of this study was to establish how important extracellular calcium influx is for the release or mechanism of action of the UDIF. Detrusor contractions (± urothelium) stimulated by carbachol or potassium chloride were compared in solutions with and without calcium. Any remaining detrusor contractions in zero calcium solutions were observed in the presence and absence of Ca2+ ATPase inhibitors, in order to deplete intracellular calcium stores.

Study design, materials and methods

Paired strips of porcine bladder dome with and without urothelium were set up under 1g tensions in gassed Krebs bicarbonate solution at 37°C. Tissues were stimulated by either a cumulative concentration-response curve or a single dose of carbachol. Alternatively, 100mM potassium chloride or 200mM Mannitol was used to contract strips. Tissue responses were compared in Krebs solution containing either 1.9mM calcium chloride or zero calcium with 1mM EGTA (calcium chelator). Potassium chloride contractions in zero calcium conditions were performed in the absence and presence of cyclopiazonic acid or thapsigargin (Ca^{2+}-ATPase inhibitors). Responses were expressed as mean tension, percentage inhibition or percentage of control and results were expressed as the mean ± sem. Paired Student's t-test was used for statistical analysis.

Results

In Krebs solution containing 1.9mM calcium carbonate, the maximum responses of detrusor strips to carbachol were inhibited by 41.05 ± 2.4% by the presence of the urothelium (n=74, p=<0.0001). Alternatively, the urothelium did not significantly inhibit contractions when the detrusor strips were stimulated by 100µM carbachol in Krebs solution containing zero calcium and 1.9mM EGTA, where intact strips contracted to 95.5 ± 5.5% of denuded maximum responses (n=4). The same phenomenon occurs when the detrusor is stimulated to contract by 100mM potassium chloride. In normal Krebs solution responses to 100mM potassium chloride were inhibited by 56.9 ± 6.2% by the presence of the urothelium (n=7, p=0.025). In Krebs solution with zero calcium (+ EGTA) the urothelium-intact strips response to 100mM potassium was 97.6 ± 9.6% of its denuded pairs response (n=18). 200mM mannitol (the same osmolarity as 100mM potassium chloride) did not stimulate a response in detrusor strips in zero calcium (EGTA) Krebs solution.

The contraction to potassium in zero calcium Krebs (+ EGTA) was not altered in the presence of the Ca^{2+}-ATPase inhibitor cyclopiazonic acid (n=4). Although, thapsigargin significantly inhibited denuded strip responses to potassium (Zero calcium EGTA Krebs solution) by 43.9 ± 10.7% (lipophillic Ca^{2+}-ATPase inhibitor, n=4, p=0.037). Ca2+-ATPase inhibitors could not abolish the potassium chloride contractions in zero calcium Krebs solution.

Krebs solution	**stimulus**	**Maximum contraction (g)**		**n**
		Denuded	Intact	
Normal Calcium	Carbachol	20.0 ± 1.2	10.9 ± 0.6***	74
	Potassium chloride	10.8 ± 2.6	4.3 ± 1.1*	7
Zero calcium (EGTA)	Carbachol	2.7 ± 0.9	2.5 ± 0.6	4
	Potassium chloride	4.4 ± 1.2	3.8 ± 0.9	8

***p=<0.0001 and *p=<0.05 indicates a significant difference from denuded values.

Interpretation of results

When there is nominal extracellular calcium available the urothelium is unable to inhibit detrusor contractions in response to carbachol or potassium chloride. This suggests that either the release of the UDIF or its mechanism of action is calcium dependant. It may be the case that the UDIF acts to inhibit L-type calcium channels, which are known to be vital for 90% of detrusor contractions in the presence of extracellular calcium. Equally an influx of extracellular calcium maybe required to release the UDIF from the urothelium.

Further investigation is required to establish what mechanism is causing the contraction to potassium and carbachol in zero calcium Krebs solution.
As an equal osmolarity solution of mannitol did not cause the detrusor to contraction it can be suggested that the potassium contraction was not due increasing the osmolarity of the Krebs solution.

Concluding message
The UDIF's mechanism of release or action requires a source of extracellular calcium. This finding may aid the identification of the UDIF and provide useful information when developing novel treatments for detrusor overactivity.

1. British journal of Pharmacology, 2000. **129**: p. 416-419.
2. Journal of Urology, 2003. **170**(5): p. 1897-1900.
3. FASEB J., 2004. **10**: p. 04-1516fje.

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