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Sui G[1], Fry C[1], Rong W[2], Wu C[1]
1. *University of Surrey,* ***2.*** *Shanghai Jiao Tong University*

DISSECTING THE NON-PURINERGIC PARACRINE MEDIATORS IN THE UROTHELIUM-MEDIATED BLADDER HYPERACTIVITY

Hypothesis / aims of study

Changes to urothelium have been reported in several bladder pathologies including bladder overactivity and most recent evidence suggests this tissue layer may exert a positive ionotropic effect in addition to sensory modulations. However, the physiological pathways underlying the urothelium-augmented detrusor activity have never been explored. This study aimed to test the hypothesis that non-purinergic paracrine mediators may be involved in the urothelium-detrusor muscle interaction by stepwise investigation on the urothelium-initiated spontaneous muscle activity.

Study design, materials and methods

Urothelium-attached detrusor muscle and urothelial sheets were isolated from guinea-pig urinary bladders (male, Dunkin-Hartley, 2-4 months old) by blunt dissection. The preparations were superfused in a HEPES-buffered physiological saline, at pH7.4, 37°C and tied to an isometric force transducer to record muscle contractions. The mediators released were measured from the superfusate sampled adjacent to the tissue preparations. The concentration of ATP was measured a luciferin-luciferase assay and prostaglandin E2 by a PGE2 immuno-assay. Data are expressed as mean±S.E.M. Student's t-tests were used to examine paired and non-paired normally distributed data sets; non-parametric equivalent tests were used for data sets of unknown distribution.

Results

Un-provoked spontaneous contractions were consistently observed in urothelial-attached detrusor muscle preparations, which were largely abolished when the urothelial mucosa was removed. Desensitising P2X receptor on detrusor muscle with alpha, beta-methylene ATP (AMBA, 10μM) attenuated (73±7% of control, n=12 bladders, $p<0.05$) but not abolished urothelium mediated muscle activity. Consistently spontaneous ATP release was detected from the urothelium-muscle preparation as well as urothelium sheets. The activity was markedly suppressed by prostaglandin EP1, 2 receptor antagonist AH6089 (13±4% of control, n=8, $p<0.05$) as well as EP1 selective antagonist SC51322 (16.8±5%, n=5, $p<0.05$). PGE2 release was detected in these urothelium-detrusor preparations (16.0±2.4ng/min/g tissue, n=12) and a higher level in urothelium sheets (51.4±4.6ng/min/g tissue, n=6, $p<0.05$ vs urothelium-detrusor preparations). Application PGE2 (0.1-1μM) also generated similar oscillatory contractile activities. Inhibition of COX activity by indomethacin (50μM) completely inhibited PGE2 release and significantly suppressed these activities (15±6% of control, n=8, $p<0.05$); another COX inhibitor diclofenac (100μM) produced a similar effect. Furthermore activation of P2Y receptors with UTP (100μM) enhanced PGE2 release (127±45% of control, n=7, $p<0.05$). Finally combined application of AMBA and indomethacin largely abolished the urothelium-mediated contractions.

Interpretation of results

The dependence on urothelium of the spontaneous contractile activity is clearly indicated by the abolition of spontaneous contractions after removal of urothelium. The attenuation of the spontaneous activity by desensitising the functional P2X receptor (absent in urothelium) on the smooth muscle and release of ATP from the urothelium support a role of ATP as a paracrine mediator in urothelium-mediated spontaneous activity. However, the inability of ABMA to block the remaining substantial contractions suggests a significant non-purinergic component in the urothelium-smooth muscle interaction. The significant suppression of the remaining activity by EP1, 2 antagonist and further by EP1 antagonist, the release of PGE2 from the urothelium-detrusor preparations, especially from the urothelial sheets, as well as the fact that PGE2 mimicked the spontaneous activity favours the notion that PGE2 is released from the urothelium and acts on receptor EP1 on the smooth muscle in a paracrine manner. The suppression of such activity and release of PGE2 from urothelium by COX inhibitors indicates that urothelial PGE2 is generated by COX. The abolition of the spontaneous activities by ABMA and indomethacin demonstrates the purino- and prostanoid- mediators as the predominant paracrine mechanism in the urothelium. The ability to release PGE2 by P2Y activation suggests a complex interaction between the two mechanisms.

Concluding message

Uroththelium-mediated, unprovoked detrusor muscle activity involves a significant non- purinergic paracrine component. This paracrine mediator is PGE2 released from the urothelium which acts on the smooth muscle EP1 receptor. Such paracrine action is regulated by COX. The release of purinergic and prostanoid mediators is the predominant paracrine mechanism in the urothelium and complex interaction exists between these two pathways.

Disclosures

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