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Abstract: 74O

Urinary bladder contractions and the influence of extracellular calcium.

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Introduction: Strong and sustained bladder contractions are vital for voiding, however, if abnormal or spontaneous contractions occur during the filling stage, bladder dysfunction may arise. One common presentation is underactive bladder, where patients present with symptoms of urgency, weak stream, nocturia, and urinary frequency. In elderly men and women with lower urinary tract symptoms, over 45% exhibit an underactive bladder, presenting this as an increasingly important and clinically relevant syndrome. However, there is a limited amount of research focussed on the mechanisms underlying underactive bladder, and therefore a paucity of treatment options available for its treatment and management (Moro et al., 2021). This emphasises the need to identify novel targets in the urinary bladder that can be used in future medications. The stimulation of some classes of G protein-coupled receptors (GPCRs) results in contractions of the urinary bladder. Of particular interest are the muscarinic, histaminergic, 5-hydroxytryptamine (5-HT), neurokinin-A (NKA), prostaglandin E2 (PGE2), and angiotensin-II (ATII) receptor systems (Phelps et al., 2022). One primary function of the GPCRs in the urinary bladder may be the modulation of calcium (Ca^{2+}) channels in the cell membranes, accommodating an influx of Ca^{2+} from extracellular fluids, and mediating a variety of physiological responses, including bladder contractions and increased pacemaker activities. **Aim**: This study aimed to determine the influence of extracellular Ca^{2+} in G protein-coupled receptor-mediated contraction of the various tissue layers of the urinary bladder. **Methods**: Urinary bladders of Large White-Landrace-Duroc pigs (6 months old, 80kg live weight) were used as the tissue in this study as they are similar in the anatomy and physiology of the human bladder. Ethical approval was not required for this study as tissues were sourced from the local abattoir after slaughter for the routine commercial provision of food. Strips of urothelium and lamina propria were isolated from the bladder wall and suspended in organ baths containing Krebs-Henseleit bicarbonate solution at 37°C and perfused with carbogen gas (95% O_2, 5% CO_2). Tissue contractions (grams) were recorded before and after the addition of a single dose of GPCR agonist in the absence and presence of 1µM nifedipine or nominally zero Ca^{2+} solution. A paired Student's two-tailed *t*-test was used to analyse results, where $p < 0.05$ was considered statistically significant. **Results**: When receptor agonists carbachol (1µM), histamine (100µM), 5-HT (100µM), NKA (300nM), PGE2 (10µM), and ATII (100nM) were added to the tissues, U&LP baseline tension increased significantly for all activated receptors ($p < 0.001$). In the presence of the L-type Ca^{2+} channel inhibitor, nifedipine, the contractions were inhibited as follows: carbachol by 54% ($n = 11$, $p < 0.01$); histamine by 45% ($n = 8$, $p < 0.05$); 5-HT by 28% ($n = 8$, $p < 0.01$); NKA by 49% ($n = 8$, $p < 0.001$); PGE2 by 29% ($n = 8$, $p < 0.05$); and ATII by 47% ($n = 8$, $p < 0.05$). In addition, in the presence of a nominally zero Ca^{2+} solution, contractions were inhibited as follows: carbachol by 39% ($n = 11$, $p < 0.01$); histamine by 46% ($n = 8$, $p < 0.05$); 5-HT by 28% ($n = 8$, $p < 0.05$); NKA by 22% ($n = 9$, $p < 0.05$); PGE2 by 32% ($n = 8$, $p < 0.05$); and ATII by 43% ($n = 8$, $p < 0.01$). When looking at the impacts of the two methods of blocking extracellular Ca^{2+} entry in the tissue, there was no significant difference between the effectiveness of inhibiting contractile activity after receptor activation. **Conclusions**: Extracellular Ca^{2+} plays an essential role across many physiological functions, and mediates not only contraction, but also key Ca^{2+}-dependent systems which could be altered in bladder disorders. This study supports the suggestion of a prominent role of extracellular Ca^{2+} for urinary bladder contractile activity. The responses obtained from muscarinic, histamine, 5-HT, NKA, PGE2 and ATII receptor activation are highly sensitive to extracellular Ca^{2+}, presenting a mechanism potentially underlying underactive bladder.

Moro, C., Phelps, C., Veer, V., Clark, J., Glasziou, P., Tikkinen, K. A. O., & Scott, A. M. (2021). The effectiveness of parasympathomimetics for treating underactive bladder: A systematic review and meta-analysis. *Neurourol. Urodynam.* **41**(1), 127-139.

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