

A NOVEL EXTRACELLULAR CALCIUM-SENSING SYSTEM IN PIG URETHRAL SMOOTH MUSCLE CELLS

Hypothesis / aims of study

Urethral smooth muscle has an important role at both filling and voiding phase, namely, is a very specific organ, which relaxes during micturition, on the other hand, contracts during voiding phase. Hence, urethral smooth muscle contracts almost all the time (>99%). Sympathetic (α) nerve is innervated and the stimulation of α adrenoceptor regulates the contraction of urethral smooth muscle leading to urethral closer. However, it is hard to consider that excitation of sympathetic nerve physiologically continues to keep the urethral closer. Recently we found the specific novel mechanism which is sensitive to extracellular calcium correlated with the force development of contraction in pig urethral smooth muscle. This force development is well reversed by nitric oxide leading to good relaxation. On the other hand, the tension force of detrusor smooth muscle was never potentiated by the extracellular calcium. These results are physiologically consistent with lower urinary tract function. Thus, the aim of this study is designed to clarify the Ca^{2+} -sensing system diversified on urethral smooth muscle.

Study design, materials and methods

Pig lower urinary tracts were obtained from the abattoir. The following protocols were performed using pig detrusor and urethral smooth muscle.

Tension Force Measurement: The intact or permeabilized (α -toxin) small strips of detrusor or urethral smooth muscle were mounted to transducer for isometric force recording. The effect of various ion channel inhibitors and Ca^{2+} store-related drugs were investigated.

Ca^{2+} -imaging study: pig urethra smooth muscle cells were cultured on coverslips and incubated with fluo-4/AM. Fluo-4 fluorescence images were obtained using a confocal-scanning microscope. The effect of extracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_{\text{out}}$) on intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_{\text{in}}$) was investigated.

Molecular Study: Quantitative PCR and Western Blot analysis were performed to detect out target proteins in pig detrusor and urethral smooth muscle. The expression at gene or protein level of Ca^{2+} -sensing receptor was investigated.

Results

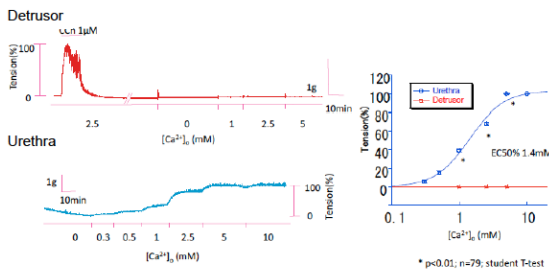
Using permeabilized tissues, the relationships between the force developments and the concentration of intracellular calcium were not significant between detrusor and urethral smooth muscles ($\text{EC}_{50} = 0.9 \text{ mM}$ and 1.1 mM , respectively). However, using intact tissues, only urethral but not detrusor smooth muscle enhanced tension force by the gradual increase of extracellular calcium ($[\text{Ca}^{2+}]_{\text{out}}$) in a concentration-dependent manner (up to 10 mM) (Fig. 1A). Various types of Ca^{2+} channel inhibitors failed to complete inhibition of the force development induced by $5 \text{ mM } [\text{Ca}^{2+}]_{\text{out}}$. Namely, N-type Ca^{2+} channel inhibitor, ω -conotoxin ($1 \mu\text{M}$) and T-type Ca^{2+} channel inhibitor, mibefradil ($10 \mu\text{M}$) slightly inhibited the $5 \text{ mM } [\text{Ca}^{2+}]_{\text{out}}$ -induced tension force by $94.1 \pm 1.2 \%$ and $90.7 \pm 1.7\%$ ($n = 6 - 8$), respectively. Further, the tension force did not diminish to $26.9 \pm 3.5 \%$ by even L-type Ca^{2+} channel inhibitor, nifedipine ($10 \mu\text{M}$) ($n = 12$). On the other hand, Ca^{2+} -activated Cl^- channel inhibitor, niflumic acid well-inhibited the $5 \text{ mM } [\text{Ca}^{2+}]_{\text{out}}$ -induced tension force by $22.0 \pm 4.7 \%$ ($n = 13$). Ca^{2+} -store related agents, $30 \mu\text{M}$ SKF96365, $10 \mu\text{M}$ cyclopiazonic acid (CPA) and $100 \mu\text{M}$ 2-aminoethoxydiphenylborate (2-APB) partially, inhibited this tension force by $65.7 \pm 4.2 \%$, $49.3 \pm 5.3 \%$ and $20.7 \pm 3.7 \%$, respectively ($n = 7 - 10$). Further, interestingly, the combination of $10 \mu\text{M}$ CPA and 10 mM caffeine completely diminished this $5 \text{ mM } [\text{Ca}^{2+}]_{\text{out}}$ -induced tension force ($n = 6$) without any channel inhibitors. (Fig. 1B).

Neither Ca^{2+} sensing receptor (CaSR) inhibitor NPS2143 ($100 \mu\text{M}$) nor activator R568 ($100 \mu\text{M}$) had no effect on this $5 \text{ mM } [\text{Ca}^{2+}]_{\text{out}}$ -induced tension force. In addition, Ca^{2+} sensing receptor-related ions, Mg^{2+} , La^{3+} and Gd^{3+} had no effect on this $5 \text{ mM } [\text{Ca}^{2+}]_{\text{out}}$ -induced tension force.

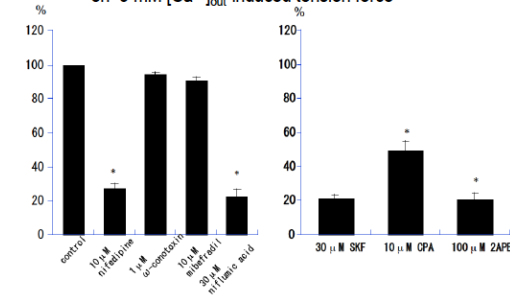
In Ca^{2+} -imaging study, the application of extracellular calcium ($[\text{Ca}^{2+}]_{\text{out}}$: 1 mM and 10 mM) reversibly induced oscillatory increase of intracellular calcium concentration ($[\text{Ca}^{2+}]_{\text{in}}$) in single pig urethral smooth muscle cells ($n = 5$). (Fig. 1C)

In the molecular study, not only quantitative PCR but also Western blot analysis indicated the predominant expression of CaSR in urethral than in detrusor smooth muscle (Fig. 1D)

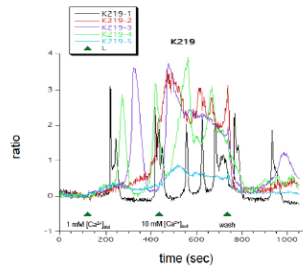
A Effect of extracellular calcium on pig detrusor and urethral smooth muscle



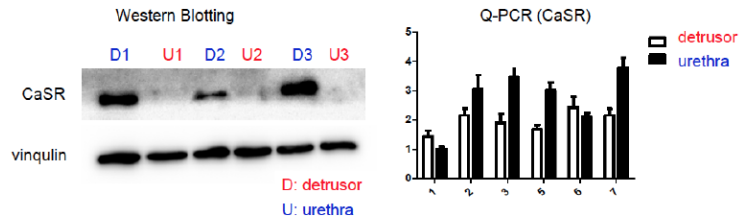
B Effect of Ion channel inhibitors & Ca²⁺-related drugs on 5 mM [Ca²⁺]_{out}-induced tension force



C Ca²⁺-imaging of pig urethral smooth muscle cells



D Molecular studies of Ca²⁺-sensing receptor in pig detrusor and urethral smooth muscle



Interpretation of results

In pig lower urinary tract, the urethral smooth muscle tone is indeed regulated by extracellular calcium different from detrusor. Although, L-type Ca^{2+} channel inhibitor, nifedipine mainly inhibited this extracellular calcium induced contraction but not completely, Ca^{2+} -activated Cl^- channel inhibitor, niflumic acid well inhibited this contraction indicating that Ca^{2+} influx through L-type Ca^{2+} channel due to the membrane depolarization is involved. Further, to maintain the tension force, not only extracellular calcium but also Ca^{2+} -release from Ca^{2+} stores are required. The molecular study strongly suggested the expression of Ca^{2+} -sensing receptor (CaSR) more abundant in urethra smooth muscle than in detrusor, however, pharmacological studies could not reveal the typical CaSR in pig urethra.

Concluding message

Our present studies clarified the extracellular calcium-sensing system in pig urethra smooth muscle to keep the smooth muscle tone leading to the stable closure of urethra at filling phase. Although pharmacological studies failed to reveal the fundamental character of CaSR, it is very likely that CaSR subtype or CaSR-like system would be involved in pig urethra. This specific novel mechanism in urethra but not in detrusor would be the most physiological function and be the target for the treatment of lower urinary tract dysfunctions.

Disclosures

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