580

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IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF CALCIUM-ACTIVATED CHLORIDE CHANNEL ANOCTAMIN-1 (ANO1) IN RAT URINARY BLADDER

Hypothesis / aims of study
Interstitial cells (ICs), analogous to the interstitial cells of Cajal in the gut, may generate phasic activity (PA) in smooth muscle tissues including the bladder (1). An established marker of the ICs is c-kit. However, recent studies have shown that anoctamin-1 (ANO1, encoded by Tmem16a), a calcium-activated chloride channel (CaCC), has a fundamental role in generation of pacemaker activity in the ICs of the gut and therefore could be used as a novel marker for these cells (2). CaCC blocking drugs such as niflumic acid (NFA) and 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) were able to alter the pacemaker activity of the ICs in the gut (3) and thus may be important modulators of these cells in other tissues. Thus, the aim of this study was to investigate whether ANO1 is expressed in the rat urinary bladder and to explore the role of NFA and NPPB in modulating the PA of the bladder tissue.

Study design, materials and methods
Bladders from male Wistar rats (p19-p23) were used. PCR was carried out on the c-DNA synthesized from total RNA isolated from the bladder dome. Primers were designed for the Rattus norvegicus ANO1 mRNA (Accession number: NM_001107564.1). PCR products were separated by electrophoresis and sequenced. Longitudinal strips (5-8mm) of intact detrusor were mounted in perspex microbaths, superfused with Krebs' solution and maintained at 37°C. Isometric tension was measured via UF1 force transducers connected to a Powerlab system using LabChart software. After 90 min of equilibration, the effect of 10µM NFA, 10µM NPPB (30min exposure) or the drug vehicle (DMSO) on basal PA was investigated by measuring the amplitude and frequency of PA. Percentage change in the amplitude and frequency of PA was calculated in presence of CaCC blockers relative to that in the absence of the blockers. All data are expressed as the mean±SEM. Statistical analysis was carried out using Student's paired t-test.

Results
ANO1 mRNA expression was found in the mucosa of the rat urinary bladder. NFA (n=12) significantly ($p<0.001$) reduced the amplitude and the frequency of basal PA by 68.4±8.8% and 78.8±8.9% respectively. NPPB (n=10) significantly reduced the amplitude of PA by 44.7±4.2% ($p<0.01$), but did not have a significant effect on the frequency of PA.

Interpretation of results
We confirm for the first time that ANO1 is expressed in mucosal layer of the rat urinary bladder. CaCC channel blockers were able to modulate the basal PA of rat bladder strips which may suggest a role of ANO1 channels in mediating the PA of ICs found in rat urinary bladder. However, the exact location and function of these channels in rat bladder requires further investigation.

Concluding message
Ano1 is expressed in the rat urinary bladder and inhibition of PA by CaCC channels may suggest an important role of the ICs in driving such activity in this species.

References
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Disclosures
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