Identification of Anoctamin (Ano1) calcium-activated chloride channels in porcine urinary bladder and characterization of their functional role in the bladder

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Introduction

Interstitial cells (ICs), analogous to the interstitial cells of Cajal of 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), influences the 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 (NA) were able to alter the pacemaker activity of the ICs in the gut and thus may be important modulators of these cells in different tissues (2).

Thus, the aim of this study was to investigate whether Ano1 is expressed in the porcine urinary bladder and to explore the role of NA in modulating the phasic activity of the bladder tissue.

Methods

Female pig (~6months old) bladders were obtained from the local abattoir, hence ethical approval was not necessary.

Molecular studies: PCR was carried out on the c-DNA synthesized from total RNA isolated from bladder mucosa (composed of the urothelium, lamina propria and a thin layer of smooth muscle (muscularis mucosa)) or denuded detrusor. Primers were designed for Sus scrofa Ano1 mRNA (Accession number: XM_003122417.2). PCR products were separated by electrophoresis and sequenced.

Functional studies: Longitudinal strips of denuded-detrusor (n=10) or mucosa (n=47) were mounted in perspex microbaths and superfused with Krebs’ solution at 37°C. Isometric tension was measured via U1 force transducers connected to Powerlab using LabChart software. Denuded-detrusor strips were superfused constantly with 0.1µM carbachol (CCh) solution to induce PA. Spontaneous basal PA was measured in mucosal strips. Increasing concentrations of NA (1-30µM) or drug vehicle (DMSO 0.01-0.3%) were added cumulatively with 10 minute exposure to each concentration. The effect of NA on spontaneous and CCh-stimulated PA was investigated by measuring the amplitude and frequency of PA. All data are expressed as the mean±SEM. Statistical analysis was carried out by using repeated measures one-way ANOVA followed by Dunnett’s post-hoc test.

Results

Molecular studies: Ano1 mRNA expression was found in mucosa and detrusor layers of porcine bladder.

![Ano1 expression in mucosa and detrusor layers](image)

Fig. 1. Expression of Ano1 in mucosa (m) and detrusor (d) of porcine bladder. Positive control amplified with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers.

Functional studies: The amplitude of basal PA in mucosa strips (n=47) was decreased by 22.3 ± 4.6% at 10µM (p<0.001) and by 26.6 ± 4.4% at 30µM (p<0.001) concentration of NA. Frequency of basal mucosal contractions was reduced by 25.0 ± 7.7% only at 30µM NA (p<0.001) (Fig. 3). Drug vehicle had no effect on PA (n = 30).

NA did not have a significant effect on the amplitude or the frequency of CCh-stimulated PA in the denuded-detrusor strips (n=10) at all concentrations. Drug vehicle had no effect on PA (n = 12).

Fig. 2. Effect of increasing concentrations of NA on the a) amplitude and b) frequency of basal PA in mucosal strips from pig bladder. p*** < 0.001 vs. 0/uni03BCM NA. Data presented as mean ±SEM (n = 47).

Fig. 3. Effect of increasing concentrations of NA on a) amplitude and b) frequency of CCh-induced PA in denuded-detrusor strips. Data presented as mean ±SEM (n = 10).

Conclusions

• We have shown for the first time that ANO1 is expressed in the pig urinary bladder.

• NA was able to modulate the basal phasic contractions of mucosal strips which may suggest a role of ANO1 channels in mediating the phasic activity of ICs found in the suburothelial layer of mucosal strips in this species.

• NA had no effect on the cholinergic-induced phasic activity of denuded-detrusor strips. This may be due to an influence of NA on PA elicited by ICs, whilst not affecting direct muscle stimulation by CCh.

References