

## IDENTIFICATION OF ANOCTAMINE (ANO1) CALCIUM ACTIVATED CHLORIDE CHANNELS IN PORCINE URINARY BLADDER AND CHARACTERIZATION OF THEIR FUNCTIONAL ROLE IN THE BLADDER

### Hypothesis / aims of study

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 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 niflumic acid in modulating the phasic activity of the bladder tissue.

### Study design, materials and methods

#### *Molecular studies:*

Primers were designed for the *Sus scrofa* ANO1 mRNA and polymerase chain reaction (PCR) carried out on the c-DNA synthesized from total cytoplasmic RNA isolated from female pig ( $\approx$ 6months old) bladders. PCR products were separated by electrophoresis and sequenced.

#### *Functional studies:*

Female pig ( $\approx$ 6months old) bladders were obtained from the local abattoir, with a warm ischemic time of  $30\pm 5$  min. For bladder strip experiments, longitudinal strips of denuded detrusor ( $n=7$ ) or mucosa ( $n=47$ ), were mounted in perspex microbaths, superfused with Krebs' solution and maintained at  $37^\circ\text{C}$ . Denuded detrusor strips were superfused constantly with  $0.1\mu\text{M}$  carbachol (CCh) solution to induce PA. The effect of increasing concentrations of niflumic acid ( $1-30\mu\text{M}$  added cumulatively, 10 min exposure for each concentration) or drug vehicle on spontaneous and CCh-stimulated PA was investigated by measuring the amplitude and frequency of PA. All data are expressed as the mean $\pm$ SEM and statistical analysis carried out using repeated measure ANOVA followed by Dunett's post hoc test

### Results

#### *Molecular studies:*

Ano1 mRNA expression was found in both mucosal and detrusor layers of the porcine urinary bladder.

#### *Functional studies:*

Spontaneous PA was detected in mucosal but not denuded detrusor strips. Niflumic acid did not have a significant effect on the amplitude or the frequency of CCh-stimulated PA in the denuded detrusor strips at all concentrations. However, the amplitude of basal PA in mucosal strips was significantly reduced ( $p<0.001$ ) with  $10\mu\text{M}$  and  $30\mu\text{M}$  niflumic acid (Fig 1A). The frequency of basal mucosal contractions was reduced significantly ( $p<0.001$ ) only at  $30\mu\text{M}$  niflumic acid (Fig 1B). The drug vehicle had no effect on PA

### Interpretation of results

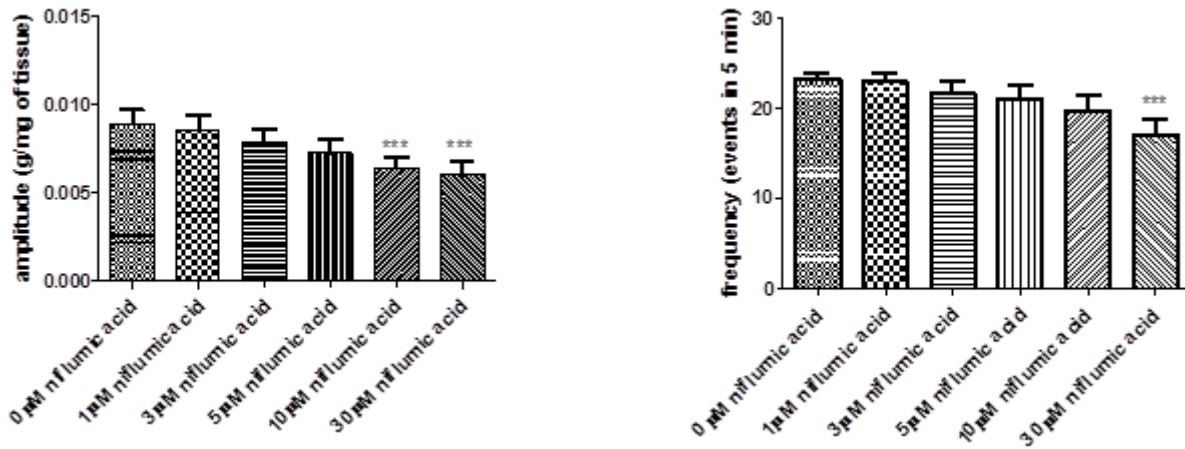
We have shown for the first time that Ano1 is expressed in the porcine urinary bladder. Niflumic acid was able to modulate the basal phasic contractions of mucosal strips which may suggest a role of ANO 1 channels in mediating the phasic activity of ICs found in the suburothelial layer of mucosal strips. However, niflumic acid had no effect on the cholinergic-induced phasic activity of denuded detrusor strips. This may be due to an influence of niflumic acid on PA elicited by ICs, while not affecting direct muscle stimulation by CCh

### Concluding message

Ano1 is expressed in the porcine urinary bladder and inhibition of PA by niflumic acid in the mucosa but not the detrusor may suggest an important role of the mucosal ICs in driving such activity in this species.

A

B



**Figure 1)** The effect of increasing concentrations of niflumic acid (1-30μM) on A) the amplitude and B) the frequency of basal PA in mucosal strips (n=47) from porcine urinary bladder. \*\*\*p<0.001 versus paired response with niflumic acid omitted. Data is presented as mean±S.E.M

#### References

1. McCloskey KD & Gurney AM (2002) J Urol, 168: 832–6
2. Hwang SJ. et al (2009). J Physio, 587:4887-4904

#### Disclosures

**Funding:** This work was supported by Early Career Researcher Grant from University of the West of England **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Pig **Ethics Committee:** Ethical approval was not required as the bladders were obtained from the local abattoir post mortem.