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EXPRESSION OF ACID-SENSITIVE RECEPTORS IN THE PORCINE URINARY BLADDER

Hypothesis / aims of study

Acidic urine is known to exacerbate the symptoms of overactive bladder in humans, by unknown mechanisms. In animal bladder, acid is a potent stimulant for release of ATP (a key signalling molecule) from rat and pig bladder mucosa [1,2]. Our aim was to quantify the expression of two families of acid sensitive receptors in porcine urinary bladders: the acid-sensing ion channels (ASICs) and the transient receptor potential vanilloid receptor 1 (TRPV1), and to localize the immunoreactivity (IR) for ASIC1.

Study design, materials and methods

Pig bladders were obtained from an abattoir, arriving on ice in the laboratory approximately 2 h after death. Any bladder appearing in any way inflamed, was discarded.

For molecular studies, separate samples (approximately 200 mg) of dome, lateral wall, neck and trigone were taken immediately, the mucosa and the detrusor were then separated and stored in 1 ml RNALater. The gene expression of ASICs and TRPV1 from neck, lateral wall, dome and trigone regions of porcine bladders was quantified using quantitative real-time polymerase chain reaction (QRT-PCR).

For immunohistochemical studies, full thickness samples of dome, lateral wall, neck and trigone were placed in Zamboni's fixative. Localisation of ASIC1 protein expression in pig bladders was determined using immunohistochemistry. Sections were preincubated with 3% H_2O_2 and 2% goat serum, then incubated overnight at room temperature with 190 µl of anti-ASIC1 (1:200, Millipore, AB5927) primary antibody in Tris buffered saline with 0.4% TritonX-100. Slides were then washed and incubated with species-specific biotinylated secondary antibody for 2 h, followed by avidin-biotinylated peroxidase complex for 1 h. Immunostaining was developed by reaction with 3, 3′-diaminobenzidine. Rat brain was used as positive control.

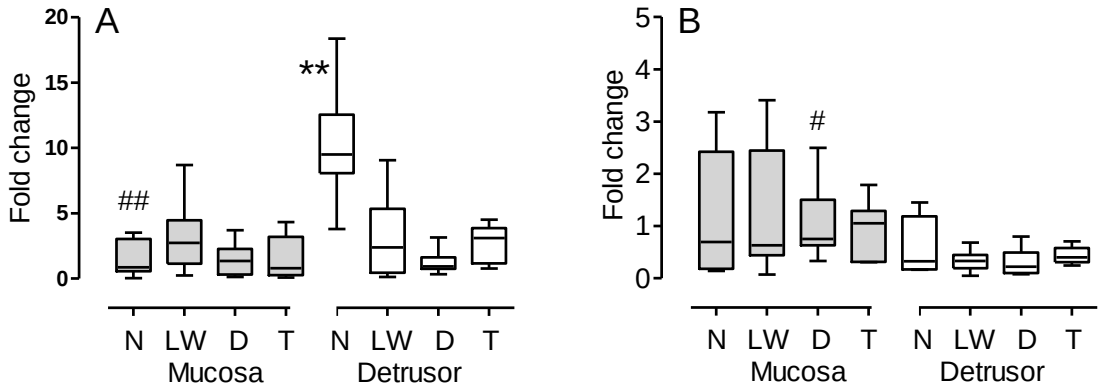

Figure 1. *Expression of mRNA for ASIC2 (A) and TRPV1 (B) in the neck (N), lateral wall (LW), detrusor (D) and trigone (T) of porcine bladder mucosa and detrusor. Data are expressed as fold change relative to the mRNA level of the housekeeping gene GAPDH and a calibrator. For ASIC2 mRNA, differences were found in the neck, where expression was > ten-fold less in the mucosa than in the detrusor neck (##, p<0.015, Kruskal-Wallis test followed by Dunn's test). ASIC2 expression in neck detrusor was also denser (**, p<0.01, Kruskal-Wallis test followed by Dunn's test) than in other detrusor regions. For TRPV1, expression in mucosal dome (G) was greater than in detrusor dome (H) (#, p<0.05, Kruskal-Wallis test followed by Dunn's test). Data are displayed as box and whiskers plots (n=7).*

Results

Molecular studies. Expression of mRNA for ASIC1, ASIC2, ASIC3 and TRPV1 was seen in all regions of the porcine bladder mucosa and detrusor (Figure 1). Expression of ASIC1 and ASIC3 was uniform for the four regions and layers, with expression (expressed as fold change relative to a pooled porcine detrusor calibrator sample and to the housekeeping gene, GAPDH). Expression of ASIC2 was 10-fold higher in the neck of detrusor compared with neck of mucosa (Figure 1A), and expression in the detrusor neck was also higher than in other detrusor regions (Figure 1A). Expression of TRPV1 mRNA in the dome region was significantly higher in mucosa than in detrusor muscle (Figure 1B).

Immunohistochemical studies. ASIC1 immunoreactivity (IR) was detected in the urothelium, lamina propria, and serosa, and absent in smooth muscle of the detrusor layer (Figure 2). Immunolabelling intensity was greatest in the urothelium, where ASIC1 IR was predominantly localised in intermediate cells (Figure 2A) of lateral wall, dome, neck and trigone of the porcine bladder (n=4). In comparison, ASIC1 IR was absent in both umbrella cells and basal cells of all regions examined. Although ASIC1 IR was slightly weaker in the dome than in other regions, no marked regional differences in ASIC1 IR in the urothelium of the dome, neck, lateral wall or trigone regions of the urinary bladder were observed. Within the lamina propria, ASIC1 IR was detected in some ganglion-like structures (Figure 2B) but not in suburothelial nerve fibres. ASIC1 IR was also present in lamina propria arteriolar smooth muscle. In suburothelial smooth muscle bundles, ASIC1 IR was intensely stained in bladder lateral wall and trigone, and only faintly in the dome and neck. In contrast, immunostaining was negligible in detrusor smooth muscle (Figure 2D).

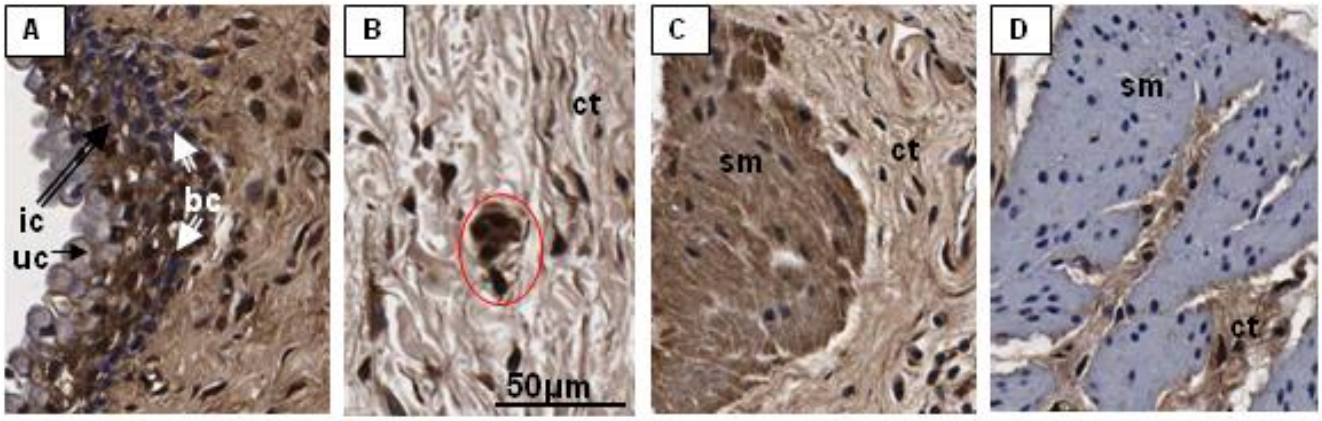

Figure 2. *Photomicrographs of porcine urinary bladder, depicting ASIC-IR (brown), counterstained with haematoxylin (blue). (A) urothelium, (B) lamina propria, (C) suburothelial smooth muscle, (D) detrusor smooth muscle. Key: uc, umbrella cells; ic, intermediate cells; bc, basal cells; ct, connective tissue; sm, smooth muscle. In (B), a ganglion-like structure is circled. Scale bar: 50 µm.*

Interpretation of results

We focused on the expression of ASICs and TRPV1 as they operate in the physiological pH range of human urine. The differential expression of ASIC2 on the neck in contrast to other bladder regions might be related to the different physiological function of the bladder neck. Unfortunately, reliable ASIC2 antibodies were not available to us.

In mouse bladders, intense ASIC1 protein expression in both umbrella cells and detrusor muscle was reported [3]. In contrast, we showed that ASIC1 was abundant in the intermediate cells and suburothelial structures and absent in umbrella cells and detrusor muscle of porcine bladder. It is unclear whether this discrepancy in ASIC1 localisation was due to species differences or to the presence of alternative spliced variants of ASIC1 (ASIC1a and ASIC1b).

Concluding message

In conclusion, this is the first study to demonstrate the gene expression of ASICs and TRPV1 in porcine urinary bladders. ASIC1-like immunoreactivity was detected in the urothelium, lamina propria, and serosa, but was negligible in detrusor smooth muscle.

References

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3. Kobayashi H, Yoshiyama M, Zakoji H, Takeda M, Araki I. (2009) BJU Int. 104: 1746-1751.

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Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**No**
Statement that no ethical approval was needed	**No ethical approval was required for these studies on pig bladder.**